Proc. Natl. Acad. Sci. USA
Vol. 87, pp. 2931–2935, April 1990
Biochemistry

Hemoglobin degradation in the malaria parasite *Plasmodium falciparum*: An ordered process in a unique organelle

(vacuoles/lysosomes/aspartic protease/hemozoin)

DANIEL E. GOLDBERG*, ANDREW F. G. SLATER, ANTHONY CERAMI, AND GRAEME B. HENDERSON

Laboratory of Medical Biochemistry, Rockefeller University, New York, NY 10021

Communicated by William Trager, January 8, 1990 (received for review November 30, 1989)

ABSTRACT **The malaria parasite *Plasmodium falciparum* uses host erythrocyte hemoglobin as a major nutrient source. We report the purification of *P. falciparum* digestive vacuoles and characterization of the degradative process therein. Vacuoles were isolated by a combination of differential centrifugation and density gradient separation. The pure vacuoles were capable of degrading hemoglobin to small fragments with a pH optimum of 5–5.5. Proteolysis in the vacuoles appears to be an ordered process, requiring an aspartic protease to clip intact hemoglobin before other proteolytic activities can function efficiently. The vacuoles do not contain other hydrolases commonly found in lysosomes and therefore appear to be unique proteolytic organelles designed specifically to degrade hemoglobin.**

During the intraerythrocytic phase of its life cycle, the malaria parasite matures within a cell in which hemoglobin is the single major cytosolic protein. While in the trophozoite stage, the parasite avidly ingests and degrades host erythrocyte hemoglobin by means of a specialized structure called a cytostome (1–5), which spans the double membrane between erythrocyte and parasite cytoplasm. Hemoglobin-containing vesicles are pinched off from the cytostome and travel to the digestive vacuole where the hemoglobin is broken down (2, 3, 5–7). The process of hemoglobin degradation releases heme, which accumulates in crystalline particles within the digestive vacuoles (8). The formation of these pigmented crystals (called hemozoin) is poorly understood.

Since the parasite has a limited capacity to synthesize amino acids *de novo* (9, 10) or to take them up exogenously (11), the hemoglobin is thought to be broken down to provide amino acids for its growth and maturation (10, 12, 13). It has been estimated that between 25% and 75% of the hemoglobin in an infected erythrocyte is degraded (14–17). Therefore, in an average patient with about 750 g of circulating hemoglobin and a heavy malaria infection at 20% parasitemia, up to 100 g of hemoglobin is utilized during a single cycle. To account for this enormous amount of protein breakdown that occurs in just a few hours of the trophozoite stage (13), we postulated that the parasite must possess an efficient and probably highly specific pathway for hemoglobin proteolysis. Previous attempts to study this process have been hampered by difficulty in purifying plasmodial proteases and by inability to define which proteases are truly involved in hemoglobin breakdown. Several putative malarial hemoglobinase activities have been described (18–27), and both cysteine and aspartic proteases have been proposed to be the major hemoglobinolytic enzyme. It has not been possible to demonstrate that any of these activities has a role in hemoglobin proteolysis *in vivo*. In order to better define the process of hemoglobin degradation that actually occurs, we have purified and characterized the digestive vacuoles from the human malarial parasite *Plasmodium falciparum*. Our data indicate that this organism has evolved a unique proteolytic organelle to degrade hemoglobin in an ordered and efficient manner. The results provide a basis for the further dissection and inhibition of the hemoglobinolytic pathway critical to this important human pathogen.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

EXPERIMENTAL PROCEDURES

Parasite Culture. *P. falciparum* clone HB-3 was cultured by the method of Trager and Jensen (28). Synchrony was maintained by sorbitol treatment (29).

[^{3}H]Hemoglobin Preparation. Erythrocytes (50–60% reticulocytes) were obtained from Sprague–Dawley rats injected with 10 mg of phenylhydrazine 3 and 5 days previously. After washing three times to remove the buffy coat, they were incubated for 2 hr with 1 mCi of L-[3,4,5-^{3}H]leucine (New England Nuclear; 6.5 mmol/Ci; 1 Ci = 37 GBq) in leucine-free medium (30). Hemoglobin was then purified by sequential anion-exchange and gel filtration chromatography (31) and its concentration was determined (32). Since hemoglobin has abundant leucines distributed throughout the molecule (33, 34), even single cleavage of a hemoglobin subunit will generate a labeled fragment that can be detected by the assays described below.

Digestive Vacuole Isolation. Three hundred milliliters of synchronized parasite culture at the late trophozoite stage, 10–15% parasitemia, was harvested and washed twice with 100 ml of phosphate-buffered saline (PBS) containing 1.5 mM magnesium chloride. The cells were incubated with 5 volumes of 5% D-sorbitol at room temperature for 10 min and centrifuged at 650 × *g* for 7 min. The supernate was mixed with 0.05 volume of 1% saponin in PBS and 0.01 volume of 50% streptomycin sulfate. After another 10 min at room temperature, the preparation was centrifuged at 1500 × *g* for 10 min. All subsequent steps were performed at 4°C. The pellet was washed with 10 ml of PBS/1.5 mM magnesium chloride/0.5% streptomycin sulfate and was resuspended in 1 ml of 0.25 M sucrose/10 mM sodium phosphate/0.5% streptomycin sulfate, pH 7.1. The suspension was triturated 10 times using a tuberculin syringe with a 27-gauge 1.24-cm needle. It was then mixed with 13 ml of 42% Percoll/0.25 M sucrose/1.5 mM magnesium chloride, pH 7.0, and centrifuged at 14,500 rpm for 40 min in a Sorvall RC2B (1.0×10^6 g·min). The Percoll self-forms a density gradient upon centrifugation. The bottom 0.5 ml of the gradient routinely consisted of a black band of material, which was harvested, diluted with 5 volumes of 0.25 M sucrose/10 mM sodium phosphate/1.5 mM magnesium chloride, pH 7.1, and centrifuged at 16,000 × *g* for 15 min. The black pellet, containing purified vacuoles, was used for morphologic and enzymatic studies.

Preparation of Vacuole Extract. The purified vacuole pellet was resuspended in 30 μl of 0.25 M sucrose/10 mM sodium phosphate/1.5 mM magnesium chloride, pH 7.1. At this point, aliquots were taken for microscopy or vacuole quan-

Abbreviations: TCA, trichloroacetic acid; EM, electron microscopy.
*To whom reprint requests should be addressed.

titation. The remainder was diluted with 3 volumes of 10 mM sodium citrate (pH 4.0) and subjected to two cycles of rapid freeze/thaw. Protein was determined by Pierce assay.

Centricon Assay for Proteolysis. Varying concentrations of enzyme extract were added to an incubation mixture containing 10 μM [^{3}H]hemoglobin (250,000 cpm/nmol), 0.2 M sodium citrate pH 5 (or pH 4 where noted), and 10 mM dithiothreitol, with or without a specific protease inhibitor, to give a final reaction volume of 50 μl. After incubation at 37°C, generally for 1 hr, the reaction was stopped by addition of 1 ml of iced 6 M guanidine hydrochloride. After 15 min on ice, the mixture was loaded onto a Centricon 10 filter (Amicon) and centrifuged at 5000 × *g* for 1 hr. One-half milliliter of filtrate was assayed for radioactivity. The assay was linear with time and protein concentration.

Trichloroacetic Acid (TCA) Assay for Proteolysis. The Centricon assay incubation mix was used with citrate/phosphate buffer at varying pH. The reaction was stopped by addition of 0.25 ml of iced, unlabeled hemoglobin (1.4 mg/ml) followed by 0.3 ml of iced 20% TCA. After 30 min on ice, the mixture was centrifuged for 15 min at 16,000 × *g* and the supernate was assayed for radioactivity.

SDS/PAGE Analysis. Human hemoglobin (Sigma) was used as substrate at a final concentration of 5 μM in the Centricon assay incubation mix. The reaction was stopped by adding 12.5 μl of 5× SDS sample buffer (35) and boiling for 3 min. Samples were then run on a 20% SDS/PAGE Phastgel (Pharmacia) which was developed with silver (36).

Microscopy. Photomicrography was done with phase-contrast optics on a Nikon Microphot-FX apparatus. Vacuole preparations were prepared for electron microscopy (EM) by the technique of Langreth *et al.* (37). Thin sections were made with a diamond knife and were scanned exhaustively at high magnification by EM to look for contaminating membranes. Five hundred fields were scanned from 10 sections of two different preparations; 42 vacuoles were observed and no contaminants were found. Low-power transmission EM photographs are not presented as further evidence of purity because the vacuoles were too sparse in the sections to be captured as a group in a single field.

Marker Enzymes. Glutamate dehydrogenase (38), cytochrome *c* oxidase (39), and acetylcholinesterase (38) were assayed as described. Results are expressed in total μmol/hr in the fraction assayed. The hemoglobin in uninfected erythrocytes and in the sorbitol lysate interfered with cytochrome *c* oxidase determinations. Therefore, only an upper limit of detection for the purified vacuole activity could be measured. β-Glucuronidase, β-galactosidase, and acid phosphatase were determined by *p*-nitrophenyl assays (40). Reactions were performed at pH 4 and pH 5.5. Presented are the results of duplicate determinations from at least two separate vacuole preparations for each activity tested.

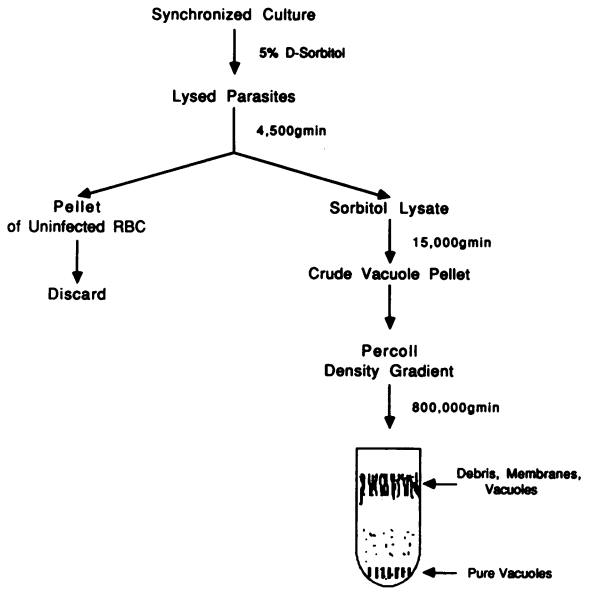

FIG. 1. Vacuole purification scheme. The purified vacuoles at the bottom of the gradient banded at a density of 1.134 (by Pharmacia density marker beads).

RESULTS

Vacuole Purification. Vacuoles were purified from synchronized cultures of *P. falciparum*-infected erythrocytes at

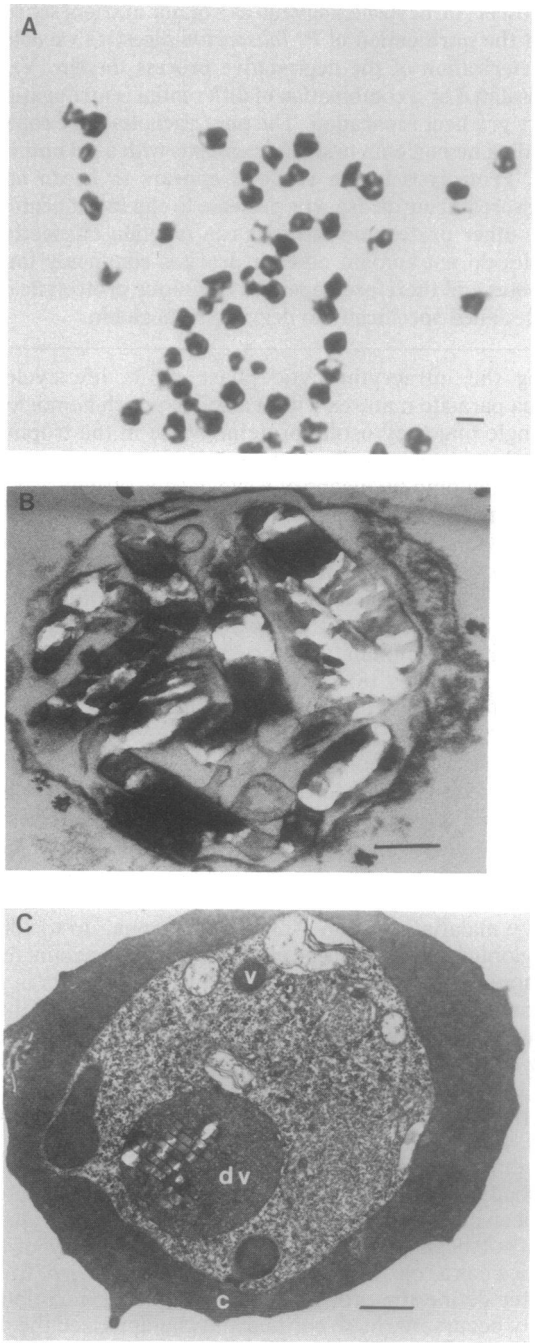

FIG. 2. Photomicrography of digestive vacuoles. (*A*) Phase-contrast micrograph of purified vacuoles. (Bar = 2 μm.) (*B*) Electron micrograph of an isolated vacuole. (Bar = 0.25 μm.) (*C*) Electron micrograph of a whole parasitized erythrocyte. (Bar = 0.5 μm.) See text for symbols.

Table 1. Marker enzyme activities

Enzyme	Uninfected erythrocytes, μmol/hr	Sorbitol lysate, μmol/hr	Vacuoles, μmol/hr	Fraction in vacuole, μmol/hr
Acetylcholinesterase	45,000	496	<0.1	<0.000002
Glutamate dehydrogenase	0.58	9.4	<0.002	<0.0002
Cytochrome *c* oxidase	—*	—*	<0.001	—*
β-Glucuronidase	4,800	<100	<0.1	<0.00002
β-Galactosidase	2,400	<200	<0.1	<0.00004
Acid phosphatase	8,000	<240	<0.25	<0.00003

*Interference from hemoglobin.

the late trophozoite stage (Fig. 1). Parasitized erythrocytes were treated with 5% D-sorbitol to lyse the mature parasites, releasing their intracellular contents. Uninfected erythrocytes remained intact and were easily sedimented by low-speed centrifugation. The supernate was mixed with a small amount of saponin and streptomycin sulfate to prevent aggregation of vacuoles with other cellular components. The vacuoles were then sedimented by higher-speed centrifugation. This crude vacuole pellet was washed and centrifuged through a Percoll gradient. Purified vacuoles migrated to the bottom of the gradient, away from membranous debris, which were found primarily at the top. The purified vacuole fraction was harvested and analyzed for purity, vacuole morphology, and intravacuolar contents. The yield of digestive vacuoles, counted by hemocytometer, was 2–3% in the four preparations assessed. This is comparable to yields from mammalian lysosome isolations (41, 42).

Phase-contrast microscopy of the purified vacuoles revealed a homogeneous preparation of yellow-black vacuoles without other membranous contaminants (Fig. 2*A*). Identical results were obtained with Wright's stained preparations (not shown). Transmission EM of isolated vacuoles revealed a population of roughly spherical, 1.5- to 2-μm (diameter) organelles, each with a classic lipid bilayer membrane surrounding a striking array of crystals (Fig. 2*B*). No other membranes or organelles were seen in the EM preparation. A micrograph of a whole parasitized erythrocyte is shown in Fig. 2*C* for comparison. In this photograph, there is a good example of a cytostome (c), involved in hemoglobin endocytosis from the erythrocyte cytoplasm, and a hemoglobin-containing vesicle (v) in the parasite cytoplasm, on its way to degradation in the digestive vacuole (dv). Note that the hemozoin crystals in the isolated vacuoles are randomly oriented, whereas those in the intact organism are consistently present in a unidirectional array.

Marker Enzymes. The purified vacuoles were assessed for possible contaminating activities and for hydrolases commonly found within mammalian lysosomes. Acetylcholinesterase (an enzyme associated with the erythrocyte membrane), glutamate dehydrogenase (associated with parasite cytoplasm), cytochrome *c* oxidase (associated with mitochondria in other organisms and reported to be present in plasmodia), and three lysosomal hydrolases (acid phosphatase, β-glucuronidase, and β-galactosidase) were assayed (Table 1). There were no detectable contaminating activities.

Table 2. Vacuole purification summary

Fraction	cpm in assay	Total activity, pmol/hr	Specific activity, pmol/hr per mg of protein	Purification, fold
Uninfected erythrocytes	12	15	0.06	—
Sorbitol lysate	328	8060	24	1
Crude vacuoles	3632	1746	246	10
Purified vacuoles	915	51	850	35

No nonproteolytic lysosomal hydrolases could be found in the purified vacuole fraction at either pH 4 or pH 5.5. The nonproteolytic hydrolases measured were all active in uninfected erythrocytes and were comparable in activity to those in crude parasitized erythrocyte extracts (not shown).

Acid Hemoglobinase Activity. In order to detect proteolytic activities capable of clipping hemoglobin, a unique assay was developed. Radiolabeled hemoglobin was incubated at 37°C with pH 5 buffer (similar results were obtained at pH 4) in the presence or absence of vacuole extract, and the reaction was stopped by addition of iced 6 M guanidine hydrochloride. The mixture was loaded onto a Centricon 10 ultrafiltration membrane (M_r cutoff, 10,000), centrifuged, and the filtrate assayed for radioactivity. Since the guanidine hydrochloride denatures hemoglobin to its monomer of M_r 16,000, even a single proteolytic cleavage anywhere in the molecule results in generation of at least one fragment that will pass through the filter and result in a positive assay.

Table 2 shows the activity of pepstatin-inhibitable acid hemoglobinase activity through the vacuole purification. Pepstatin is a specific aspartic protease inhibitor. The purification of aspartic protease activity in the isolated vacuoles was at least 35-fold relative to the sorbitol lysate. This figure may be an underestimate, since the initial specific activity is inflated by contaminating nonvacuolar proteases. Uninfected erythrocytes had minimal aspartic protease activity.

Protease Inhibitors. Hemoglobin proteolysis in the purified vacuoles was assessed in the presence of pepstatin or E-64 (a specific inhibitor of cysteine proteases). Pepstatin inhibited nearly all clipping of hemoglobin, whereas E-64 partially blocked generation of hemoglobin fragments (Table 3). Another cysteine protease inhibitor, leupeptin, gave results similar to those obtained with E-64 (not shown). Assays were done at pH 5 to simulate the reported pH of the digestive vacuoles *in vivo*. At pH 4 the dominance of pepstatin-inhibitable proteolysis was even more marked—92% of clipping was pepstatin-inhibitable and 6% was E-64-inhibitable.

The products of the above incubations were analyzed by SDS/PAGE (Fig. 3). In the absence of inhibitors a specific pattern of fragments was obtained reproducibly (lane a). Inhibitors of cysteine, serine, and metalloproteases were without effect (lane b). Addition of pepstatin to the inhibitor cocktail abolished all apparent proteolysis (lane c). When pepstatin was the only inhibitor present, the characteristic fragment pattern was not observed, though a faint smear on the gel indicates that a small amount of less specific proteolysis had occurred (lane d).

Table 3. Effect of specific protease inhibitors on hemoglobin proteolysis in vacuole extract

Inhibitor	Activity, cpm/hr per ml	% inhibition
None	11,800	—
Pepstatin (10 μM)	2,500	79
E-64 (20 μM)	8,200	30

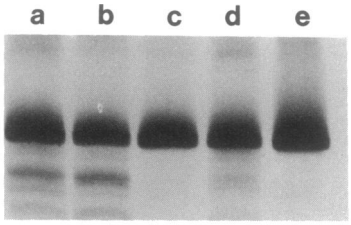

FIG. 3. SDS/PAGE analysis of hemoglobin breakdown products. Hemoglobin was incubated with purified vacuole extract in the presence or absence of inhibitors at 37°C for 1 hr. Lanes: a, no inhibitor; b, 1 mM phenylmethylsulfonyl fluoride/1 mM 1,10-phenanthroline/20 μM E-64; c, same inhibitors as in lane b with 10 μM pepstatin added; d, 10 μM pepstatin alone; e, no enzyme control.

The ability of the purified vacuoles to digest hemoglobin to small pieces was assessed by measuring production of TCA-soluble fragments. After the standard incubation with [^{3}H]hemoglobin, carrier hemoglobin was added and TCA precipitation was performed. TCA-soluble radioactivity was assayed, and results are shown in Table 4. Pepstatin was able to block almost 70% of hemoglobin degradation to small fragments, whereas E-64, phenylmethylsulfonyl fluoride and 1,10-phenanthroline had more modest effects.

pH Dependence. The pH optimum for vacuolar digestion of hemoglobin was determined by assaying ability of the purified vacuoles to generate TCA-soluble fragments at various pH values (Fig. 4). There is a broad maximum of hemoglobin degradation between pH 4.5 and 5.5; a dramatic, reproducible decline in activity occurs when the pH is raised to 6.0.

DISCUSSION

By differential centrifugation and Percoll density gradient separation, we have been able to isolate digestive vacuoles from *P. falciparum* free of detectable contaminants. There is a paucity of reliable organellar markers for plasmodia, so the assessments of purity rely primarily on analysis by EM. Choi and Mego (43) have previously prepared iron oxide granule-loaded vacuoles from *P. falciparum*. Interestingly, they showed that the parasites are able to take up these granules from erythrocyte cytoplasm into the digestive vacuoles, suggesting a bulk phase process of ingestion. Unfortunately, the presence of iron oxide precluded EM assessment of morphology and purity of the vacuole preparation.

We cannot be sure that our isolated digestive vacuoles are a fully representative population. Nevertheless, the purified vacuoles are capable of performing their putative cellular function—the catabolism of hemoglobin. We have characterized this process from several different aspects. Ultrastructurally, the vacuoles contain the end product of hemoglobin degradation, hemozoin pigment. It is curious that photographs of intact parasites show pigment crystals lined up along a single axis, whereas photographs of isolated vacuoles repeatedly show a disordered array. This may merely be an artifact of isolation or fixation. Alternatively, considering the paramagnetic properties of the iron in hemozoin (44), a reasonable hypothesis is that the pH gradient

Table 4. Effect of specific protease inhibitors on hemoglobin degradation to TCA-soluble fragments by vacuole extract

Inhibitor	TCA-soluble product, cpm per assay	% inhibition
None	633	—
Pepstatin	211	67
E-64	429	32
Phenylmethylsulfonyl fluoride	408	36
1,10-Phenanthroline	524	17

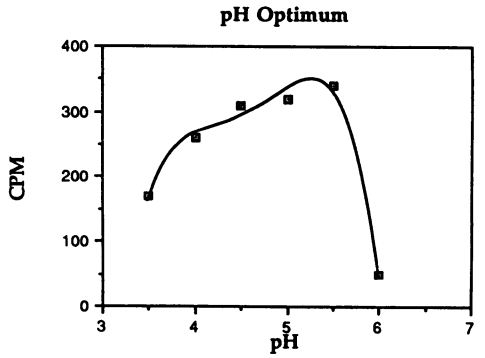

FIG. 4. pH optimum for degradation of hemoglobin to small fragments by vacuole extract. Extract was incubated with [^{3}H]-hemoglobin at 37°C for 1 hr. Carrier hemoglobin was added and TCA-soluble radioactivity was measured. Shown are results of a representative experiment, with all points averages of duplicates.

across the vacuolar membrane establishes an electromotive force across the vacuole that causes the pigment crystals to align within the magnetic field so generated. During isolation, the factors needed to maintain a transmembrane pH gradient may be lost, and the crystals would become disordered.

The isolation of digestive vacuoles has allowed us to glimpse beyond the jumble of intracellular proteases and to begin to define specific components of hemoglobin catabolism within the vacuoles. It has until now been impossible to determine which of the various activities previously described are actually present in the vacuole where hemoglobin is digested. Furthermore, it has proved difficult to purify these enzymes fully because, as the enzymes became purer, activity was lost (24, 25). We would suggest that a likely explanation is that the TCA solubility assay used in these studies is insensitive to endoproteolytic clipping so that, as the proteases that generate smaller fragments were purified away, activity in their assays disappeared.

We have studied proteolytic events in the vacuoles by using three different measures of proteolysis: breakdown to small fragments by assaying generation of TCA-soluble peptides and amino acids; production of large proteolytic intermediates by SDS/PAGE analysis; and generation of large and small fragments by using a Centricon filter assay. This last assay is a rapid system that can detect a single clip in the hemoglobin molecule but will also measure further breakdown to smaller pieces. We believe that by using *in vivo*-labeled hemoglobin, or (for the gel assay) native unlabeled hemoglobin, we provide a more natural substrate than those used in previous studies. There, acetylated or methylated protein has been used, the procedures for synthesis of which unavoidably alter the hemoglobin structure. We used labeled rat hemoglobin because of the ability to obtain a high reticulocytosis in the animals for labeling purposes. When SDS/PAGE analysis was carried out, results were similar with rat or human hemoglobin as substrate (not shown).

The results of our three assessments of hemoglobin proteolysis suggest that there are multiple and diverse proteases at work in the vacuoles. The initial event in breakdown appears to be generation of large fragments by aspartic protease activity. There are three lines of evidence for this. (*i*) Incubation of hemoglobin with vacuole extract results in production of several polypeptide pieces seen on SDS/PAGE analysis. This clipping is abolished by addition of pepstatin to the reaction. (*ii*) After such an incubation, 80% of the proteolysis detected in the Centricon assay is inhibited by pepstatin. (*iii*) The generation of small, TCA-soluble fragments can also be largely inhibited by pepstatin, but to a lesser extent than in the previous assays. This is because production of TCA-soluble material requires action of initial clipping enzymes in combi-

nation with more extensive proteolysis by secondary endo- and exopeptidases. Thus, the TCA assay only detects aspartic endopeptidase activity when its products are cleaved further by other enzymes. It was therefore possible to inhibit substantial amounts of activity in the TCA assay by addition of other classes of protease inhibitors. The data generated suggest the presence of cysteine, serine, and metalloproteases that function after aspartic protease action. Clearly, there is some proteolysis that still occurred in the presence of pepstatin. We propose that initial action of an aspartic protease is required for efficient but not exclusive proteolysis. Previous studies have shown that cysteine protease inhibitors incubated with *P. falciparum* cultures block hemozoin accumulation and arrest cell growth (27). It may well be that a cysteine protease is required for release of heme from the partially degraded globin chain. Alternatively, the protease inhibitors may be functioning by disrupting an unrelated essential cellular function.

A calculation of hemoglobin degradation rate per vacuole provides an approximate measure of the degree to which physiologic conditions have been simulated by our *in vitro* system. In our assays, an activity of 51 pmol/hr was found (Table 2), using 780,000 vacuoles for the extract. This calculates to 0.06 fmol/hr per vacuole. Others have estimated hemoglobin breakdown in the plasmodium-infected erythrocyte to be 25–75% (see Introduction). An average erythrocyte contains 0.5 fmol of hemoglobin. Thus, the vacuolar extract is capable of breaking down half of the erythrocyte hemoglobin in a few hours if it continues at the initial rate throughout the incubation. This estimated time is certainly within approximation of the actual amount of time it takes a plasmodial trophozoite to degrade hemoglobin.

The pH curve of proteolysis depicted in Fig. 4 is striking in its steep decline above pH 5.5. The pH of the plasmodium digestive vacuole has been measured to be between 5.0 and 5.4 (45, 46). In addition, pharmacologic amounts of chloroquine sufficient to kill sensitive organisms raise the intravacuolar pH by about 0.5 unit within minutes (45). Our data suggest that such a rise in intracellular pH would be sufficient to reduce the rate of hemoglobin breakdown drastically, thereby depriving the parasite of essential nutrients during its period of critical growth. The marked pH dependence of hemoglobin digestion, then, may provide a basis for the toxicity of chloroquine to malaria organisms.

The absence of detectable nonproteolytic lysosomal hydrolases in the vacuoles is significant. Though it is possible that such enzymes are present but not detected by our assay, this seems unlikely. Hydrolases were sought at a pH where their mammalian counterparts have good activity and at a pH close to that estimated to exist in the plasmodial digestive vacuole. More likely, the parasites do not need to degrade other macromolecules to sustain growth and development. Certainly, they obtain many of the nutrients they need directly from their host cell. The absence of typical lysosomal phosphatase and glycosidases indicates that the digestive vacuoles of *P. falciparum* are specialized organelles that have evolved with the primary purpose of degrading hemoglobin.

With the purification of functionally active *P. falciparum* digestive vacuoles, it is now possible to dissect the process of hemoglobin breakdown therein. We have purified a vacuolar aspartic protease and begun characterization of its kinetic properties and specificity (unpublished data). Hemoglobin catabolism in malaria organisms appears to be a metabolic pathway rather than a random process in a sack of proteases.

We thank Jaime Zung, Helen Shio, and Elena Sphilas for help with EM; Jerapan Krungkrai for providing rat reticulocytes; Jaime Zung and G. S. Gill for providing parasites; and William Trager and David Luck for helpful discussions. This work was supported by Grant RF 88062 from the Rockefeller Foundation.

1. Rudzinska, M. A. & Trager, W. (1957) *J. Protozool.* **4,** 190–199.
2. Rudzinska, M. A., Trager, W. & Bray, R. S. (1965) *J. Protozool* **12,** 563–576.
3. Aikawa, M., Hepler, P. K., Huff, C. G. & Sprinz, H. (1966) *J. Cell Biol.* **28,** 355–373.
4. Aikawa, M. (1971) *Exp. Parasitol.* **30,** 284–320.
5. Slomianny, C., Prensier, G. & Vivier, E. (1982) *Mol. Biochem. Parasitol. Suppl.* **V,** 695.
6. Theakston, R. D. G., Fletcher, K. A. & Maegraith, B. G. (1970) *Ann. Trop. Med. Parasitol.* **64,** 63–71.
7. Yayon, A., Timberg, R., Friedman, S. & Ginsburg, H. (1984) *J. Protozool.* **31,** 367–372.
8. Sherman, I. (1979) *Microbiol. Rev.* **43,** 453–494.
9. Ting, I. P. & Sherman, I. W. (1966) *Comp. Biochem. Physiol.* **19,** 855–869.
10. Sherman, I. W. (1977) *Bull. W.H.O.* **55,** 265–276.
11. Pollet, H. & Conrad, M. E. (1968) *Proc. Soc. Exp. Biol. Med.* **127,** 251–253.
12. Sherman, I. W. & Tanigoshi, L. (1970) *Int. J. Biochem.* **1,** 635–637.
13. Zarchin, S., Krugliak, M. & Ginsburg, H. (1986) *Biochem. Pharmacol.* **35,** 2435–2442.
14. Ball, E. G., McKee, R. W., Anfinsen, C. B., Cruz, W. O. & Geiman, Q. M. (1948) *J. Biol. Chem.* **175,** 547–571.
15. Morrison, D. B. & Jeskey, H. A. (1948) *J. Nat. Malar. Soc.* **7,** 259–264.
16. Groman, N. B. (1951) *J. Infect. Dis.* **88,** 126–150.
17. Roth, E. F., Brotman, D. S., Vanderberg, J. P. & Schulman, S. (1986) *Am. J. Trop. Med. Hyg.* **35,** 906–911.
18. Cook, L., Grant, P. T. & Kermack, W. O. (1961) *Exp. Parasitol.* **11,** 372–379.
19. Levy, M. R. & Chou, S. C. (1973) *J. Parasitol.* **59,** 1064–1070.
20. Levy, M. R., Siddiqui, W. A. & Chou, S. C. (1974) *Nature (London)* **247,** 546–549.
21. Hempelmann, E. & Wilson, R. J. M. (1980) *Parasitology* **80,** 323–330.
22. Aissi, E., Charet, P., Bouquelet, S. & Biguet, J. (1983) *Comp. Biochem. Physiol. B* **74,** 559–566.
23. Gyang, F. N., Poole, B. & Trager, W. (1983) *Mol. Biochem. Parasitol.* **5,** 263–273.
24. Sherman, I. W. & Tanigoshi, L. (1983) *Mol. Biochem. Parasitol.* **8,** 207–226.
25. Vander Jagt, D. L., Hunsaker, L. A. & Campos, N. M. (1986) *Mol. Biochem. Parasitol.* **18,** 389–400.
26. Sato, K., Fukabori, Y. & Suzuki, M. (1987) *Zentralbl. Bakteriol. Mikrobiol. Hyg. Ser. A* **264,** 487–495.
27. Rosenthal, P. J., McKerrow, J. H., Aikawa, M., Nagasawa, H. & Leech, J. H. (1988) *J. Clin. Invest.* **82,** 1560–1566.
28. Trager, W. & Jensen, J. B. (1976) *Science* **193,** 673–675.
29. Lambros, C. & Vanderberg, J. P. (1979) *J. Parasitol.* **65,** 418–420.
30. Huisman, T. H. J. & Jonxis, J. H. P. (1977) *The Hemoglobinopathies* (Dekker, New York), pp. 192–194.
31. Fagan, J. M., Waxman, L. & Goldberg, A. L. (1986) *J. Biol. Chem.* **261,** 5705–5713.
32. Tentori, L. & Salvati, A. M. (1981) *Methods Enzymol.* **76,** 707–713.
33. Chua, C. G., Carrell, R. W. & Howard, B. H. (1975) *Biochem. J.* **149,** 259–269.
34. Garrick, L. M., Sloan, R. L., Ryan, T. W., Klonowski, T. J. & Garrick, M. D. (1978) *Biochem. J.* **173,** 321–330.
35. Albert, K. A., Nairn, A. C. & Greengard, P. (1987) *Proc. Natl. Acad. Sci. USA* **84,** 7046–7050.
36. Wray, W., Boulikas, T., Wray, V. P. & Hancock, R. (1981) *Anal. Biochem.* **118,** 197–203.
37. Langreth, S. G., Jensen, J. B., Reese, R. T. & Trager, W. (1978) *J. Protozool.* **25,** 443–452.
38. Vander Jagt, D. L., Intress, C., Heidrich, J. E., Mrema, J. E. K., Rieckmann, K. H. & Heidrich, H. G. (1982) *J. Parasitol.* **68,** 1068–1071.
39. Scheibel, L. W. & Miller, J. (1969) *J. Parasitol.* **55,** 825–829.
40. Kodolvsky, O. & Palmeiri, M. (1971) *Biochem. J.* **125,** 697–701.
41. Maunsbach, A. B. (1974) *Methods Enzymol.* **31,** 330–339.
42. Wattiaux, R., Wattiaux-de Coninck, S., Ronveaux-Dupal, M. F. & Dubois, F. (1978) *J. Cell Biol.* **78,** 349–368.
43. Choi, I. & Mego, J. L. (1988) *Mol. Biochem. Parasitol.* **31,** 71–78.
44. Fairlamb, A. H., Paul, F. & Warhurst, D. C. (1984) *Mol. Biochem. Parasitol.* **12,** 307–312.
45. Krogstadt, D. J., Schlesinger, P. H. & Gluzman, I. Y. (1985) *J. Cell Biol.* **101,** 2302–2309.
46. Yayon, A., Cabantchik, Z. I. & Ginsburg, H. (1984) *EMBO J.* **3,** 2695–2700.