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Research Note

The Influence of C3 Component on the Hydrolysis of Human Hemoglobin by a Hemoglobin Protease from Lung Fluke Worms

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It is well known that a hemoglobin protease has been found in some parasitic helminths. In recent years, Hamajima and Yamagami (1981) revealed that the enzyme activity was inhibited by some antiproteases of human plasma. Therefore, we have thought it worthwhile to elucidate the effects of some proteins from human plasma on the hemoglobin protease activity of *Paragonimus westermani* in connection with the host-parasite relationship. Thus, this paper reports the influence of C3 component and C3 activator on the hydrolysis of human hemoglobin by the protease of lung fluke worms.

The adult worms of *P. westermani* (triploid type) used in the experiments were obtained from worm cysts in lungs of dogs 10 months after inoculation with metacercariae isolated from *Eriocheir japonicus* collected on Tsushima Is., Japan. α_1-Antitrypsin for affinity chromatography was purchased from Sigma Chemical Co. C3 component and C3 activator used in the experiments were kindly supplied by Prof. N. Heimburger and Dr. H. Karges of the Behring Institute. Sepharose 4B, DEAE-cellulose and CM-cellulose were obtained from Pharmacia Fine Chemicals, Whatman Biochemicals and Serva Feinbiochemica GmbH, respectively. All chemicals employed were of the highest purity. Hemoglobin protease of the lung fluke was purified by affinity chromatography (Hamajima and Yamagami, 1981). Protein concentration was determined by the method of Lowry *et al.* (1951) with bovine serum albumin as a standard. Assay of proteolytic activity was basically performed by the method reported by Anson (1939). Purified enzyme (3.67 units per assay mixture) was used in the experiment. The enzyme (20 μl) was treated with 10 μl C3 component or C3 activator (10 μg) in 0.05 M phosphate buffer, pH 6.0, in a final volume of 30 μl for 30 min at 0 C, and then 75 μl hemoglobin (500 μg) in 0.2 M acetate buffer, pH 4.0, in a final volume of 105 μl was added to the reaction mixture. The reaction mixture was incubated for 40 min at 37 C. Then trichloroacetic acid was added to a final concentration of 2.38%. The mixture was allowed to stand for 60 min at 0 C and then centrifuged. The liberated peptide in the supernatant was measured by the methods of Lowry *et al.* (1951). The absorbance was determined at 660 nm with a Beckman spectrophotometer. Evaluation of the influence of C3 component or C3 activator on the protease was based on increase in the hydrolysates from substrate. The activity of the enzyme was expressed as ΔOD at 660 nm. One unit of the enzyme activity was defined as the activity producing an increase of 1.0 in absorbance at 660 nm per

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Table 1 The influence of C3 component on the hydrolysis of human hemoglobin by a hemoglobin protease of *Paragonimus westermani* adult worms

Protein	ΔOD ($\times 10^{-3}$) at 660 nm Mean±SD	Relative activity (%)	P
Control	188±0.0	100	—
C3 activator	192±1.9	101.9	NS
C3 component	314±2.1	166.8	<0.001

Each value represents the mean of triplicate determinations with the standard deviations (SD). Probability (P) is expressed in P-value. NS indicates not significant by the t-test.

minute per ml.

Table 1 shows the influence of C3 component and C3 activator from human plasma on the hydrolysis of human hemoglobin by the purified enzyme. The extent of hydrolysis of hemoglobin by the enzyme was increased above the level of the control when C3 component was added as an activator. The differences are statistically significant. However, the C3 component in the absence of the enzyme did not increase the hydrolysates above the level of a blank reaction system in the absence of the enzyme and the C3 component. On the contrary, C3 activator did not stimulate formation of the hydrolysates.

Lung flukes injure various tissues and organs when penetrating and feeding during their migrations to the lungs (Yokogawa *et al.*, 1960). Human plasma contains C3 component and C3 activator. Thus, activation of hydrolysis of hemoglobin with the protease of the worm by some proteins from human plasma is of interest from the point of view of the susceptibility of the host to the worms and the pathology produced. In the present study, formation of the hydrolysates of hemoglobin with the purified enzyme was appreciably stimulated by C3 component. From these results, it would appear that C3 component activated the enzyme that hydrolyzes hemoglobin. It seems probable, therefore, that C3 component is in opposition to antiproteases for activity of the enzyme (Hamajima and Yamagami, 1981; Hamajima *et al.*, 1982).

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短　報

肺吸虫ヘモグロビン分解酵素による人ヘモグロビンの水解に対する C3 Component の影響

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ウエステルマン肺吸虫 (3n 型) の終宿主における寄生現象を生化学面から解明するため，本肺吸虫成虫におけるヘモグロビン分解酵素による人ヘモグロビンの水解に対する C3 component および C3 activator の影響を検討した．その結果，C3 component は本水解を刺激した．