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Erythrocyte aggregation under high pressure studied by laser photometry and mathematical analysis

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ABSTRACT

The effects of hydrostatic pressure on erythrocyte aggregation have been studied by laser photometry and analysis based on a phenomenological theory. Samples were prepared by suspending swine erythrocytes in their own plasma. A high-pressure vessel consisting of a stainless-steel block with a hole to hold a sample cell and two sapphire windows to allows the passage of a He–Ne laser beam was used in the experimental setup. The suspension was stirred at 1500 rpm to disperse the erythrocytes homogeneously. Immediately after reducing the stirring rate from 1500 rpm to 300 rpm, the transmitted light intensity (I) was recorded every 10 ms under a high pressure of 40–200 MPa. The value of I increased with time (t) owing to erythrocyte aggregation. From the phenomenological theory, the equation $\Delta I(t) = \Delta I_{eq}\left[1 - e^{-Kt}/\left(1 - B\left(1 - e^{-Kt}\right)\right)\right]$ was derived for the change in the transmitted light intensity (ΔI) due to erythrocyte aggregation, where ΔI_{eq} is the transmitted light intensity in the steady state, K is a time constant and B is a constant that represents the ratio of the number of interaction sites on erythrocyte aggregates at time t to that in the steady state. The observed time courses of ΔI obtained at all pressures could be closely fitted to the theoretical equation. ΔI_{eq} roughly increased with increasing pressure. On the other hand, K and B abruptly decreased above 120 MPa. The growth rate of aggregates decreased above 120 MPa. These results suggest a change in the mechanism of erythrocyte aggregation at approximately 120 MPa. We discuss the physical meaning of the parameters.

1. Introduction

When erythrocytes are suspended in a plasma or other macromolecular solutions, they form face-to-face aggregates called rouleaux, which are easily broken up by mechanical shearing. It has been pointed out that rouleaux formation affects in vivo blood flow [1]. There are two models for erythrocyte aggregation: the bridging model [2] and the depletion model [3]. The mechanism of erythrocyte aggregation is yet to be fully elucidated, although recent works have tended to favor the depletion model [4,5]. The degree of aggregation depends on the physical environment, such as the temperature and pressure, as well as the chemical properties of erythrocytes and their suspending medium [6–8]. There have been some reports on the effects of temperature effects on erythrocyte aggregation [9,10]. Whereas pressure together with temperature is an important thermodynamic variable for the study of erythrocyte aggregation as well as for a variety of applications, there has been insufficient study of the effects of pressure on this phenomenon because of experimental difficulties. A high pressure can be used for the preservation and sterilization of blood. Although it is desirable to preserve blood at subzero temperatures, the process of freezing and thawing causes the hemolysis of erythrocytes. On the other hand, the freezing point of water is lowered under hydrostatic pressure to −20 °C at 200 MPa [11]. Therefore, the freezing point can be depressed to enable the non-freezing preservation of blood [12]. Another potential application is the inactivation of microorganisms in blood, particularly pathogenic viruses. The infectivity of various types of viruses has been found to be greatly reduced by high-pressure treatment [13–16].

Aggregation behavior has been conventionally assessed by the erythrocyte sedimentation rate (ESR) and microscopic observation [17]. In a previous study, we found that the ESR increased with increasing hydrostatic pressure up to 200 MPa [18,19]. However, ESR measurement only provides the total amount of supernatant plasma due to the sedimentation of aggregated erythrocytes, although this is implicitly related to the aggregability of erythrocytes. Measurements of transmitted or forward-scattered light have been utilized to study the kinetics of aggregate formation [20]. In this study, we have developed a laser photometric system to investigate the aggregation kinetics under high hydrostatic

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Nomenclature

$I(t)$	Transmitted light intensity at time t [V]
$J_j(t)$	Decrease in the number of individual erythrocytes per unit time from the jth aggregate at time t [1/s]
$J_{tot}(t)$	Total decrease in the number of individual erythrocytes per unit time at time t [1/s]
$k^{(-)}$	Kinetic coefficient of attachment [m/s]
$k^{(+)}$	Kinetic coefficient of detachment [m^2/s]
$n_{ag}(t)$	The number of erythrocyte aggregates at time t
$n(t)$	The number of individual erythrocytes in the suspension at time t
n_{tot}	The number of individual erythrocytes in the initial state
n_{eq}	The number of individual erythrocytes in the final steady state
$N_j(t)$	The number of erythrocytes in the j-th erythrocyte aggregate at time t
$N(t)$	The total number of erythrocytes making up the aggregates
$S_j(N_j(t), t)$	Attachment area of the jth aggregate at time t [m^2]
$S_{tot}(t)$	Total attachment area at time t [m^2]
S_{tot}^{eq}	Total attachment area in the final steady state [m^2]
S_{tot}^{in}	Total attachment area in the initial state [m^2]
v_0	Volume of an erythrocyte [m^3]
V	Volume of the suspension [m^3]
$\Omega(t)$	Total aggregate volume at time t [m^3]
Ω_{in}	Total aggregate volume soon after the transient behavior has finished [m^3]
Ω_{eq}	Total aggregate volume in the final steady state [m^3]

pressures. Using this system, the effects of hydrostatic pressure on the erythrocyte aggregation process have been studied over a wide pressure range with pressures of up to 200 MPa. Swine blood were used in this experiments. The erythrocyte aggregation level in swine blood is known to be similar to that of normal human blood [21]. The transmitted light intensity is theoretically related to the aggregation of erythrocytes and characteristic parameters. By fitting the data to the theoretical equation, we discuss the effects of a high hydrostatic pressure on erythrocyte aggregation.

2. Materials and method

Fresh blood was obtained from healthy swine ($n = 6$) and anticoagulated with EDTA–2 K (1 mg/ml). The blood sample was centrifuged at 1670 × g for 10 min to collect the supernatant plasma and remove the buffy coat. Packed erythrocytes were resuspended in their own plasma at a hematocrit (Ht) of 2.0% as the final erythrocyte suspension (erythrocytes in plasma). An erythrocyte suspension in phosphate buffered saline solution (155 mM NaCl, 3.9 mM K_2HPO_4, 0.7 mM KH_2PO_4, pH 7.4) was also prepared at Ht = 2.0% as a non-aggregative control sample (erythrocytes in saline).

Fig. 1 shows a diagram of the experimental setup. A 15 mW He–Ne laser beam with 0.8 mm in diameter was used as a light source. The high-pressure vessel was made of stainless steel and equipped with a rectangular hole to hold a sample cell of the dimensions 10 × 10 × 45 mm and two sapphire windows of 10 mm diameter to allow the passage of the laser beam. Hydrostatic pressure was generated through water using a screw-type pump. The erythrocyte suspension was placed in the sample cell, which had a cylindrical magnetic stirring rod in it. The sample cell was tightly sealed with a plastic film without trapping air bubbles and placed in the high-pressure vessel, which was immersed in a water bath whose temperature was controlled at 25.0 ± 0.1 °C. The erythrocyte suspension in the high-pressure vessel was mixed at the desired stirring rate using a strong magnetic stirrer. The transmitted light that passed through the erythrocyte suspension was amplified and then detected by a silicon photodiode. The transmitted light intensity converted into a voltage was continuously recorded by a personal computer through a digital multimeter.

The erythrocyte suspension was pressurized at a rate of 20 MPa/min and then maintained at the desired pressure. To homogeneously disperse the erythrocytes in the plasma, the erythrocyte suspension was stirred at 1500 rpm for 60 s. Immediately after reducing the stirring rate from 1500 rpm to 300 rpm, the transmitted light intensity (I) was measured every 10 ms; note that when stirring at 300 rpm, neither sedimentation nor hemolysis occurs. The measurements were carried out on samples from six different individuals.

3. Relationship between transmitted light intensity and aggregation process

3.1. Application of phenomenological theory to aggregation process

Let us assume that the nuclei of the erythrocyte aggregates are generated during the transient process in which the stirring rate is reduced from 1500 rpm to 300 rpm and that the aggregates grow by the attachment of individual erythrocytes to the nuclei after the short transient time. We choose the time at which the transient process is completed as the origin of the elapsed time t. We also assume that nucleation from individual erythrocytes does not occur after the transient time; the number of aggregates is changed by the fusion and division of the aggregates. Let the number of erythrocyte aggregates at time t be denoted by $n_{ag}(t)$ and the number of erythrocytes in the j-th erythrocyte aggregate $\left(j = 1, 2, \cdots, n_{ag}(t)\right)$ at time t be denoted by $N_j(t)$. We also assume that the area of the j-th aggregate to which individual erythrocytes can attach is a function of $N_j(t)$. We denote the "attachment" area of the j-th aggregate by $S_j\left(N_j(t), t\right)$. The volume of the suspension is denoted by V and the number of individual erythrocytes in the suspension is denoted by $n(t)$.

Here we consider the decrease in the number of individual erythrocytes in the suspension. The number of individual erythrocytes is changed by their attachment to and detachment from the aggregates. The number of individual erythrocytes attaching to the

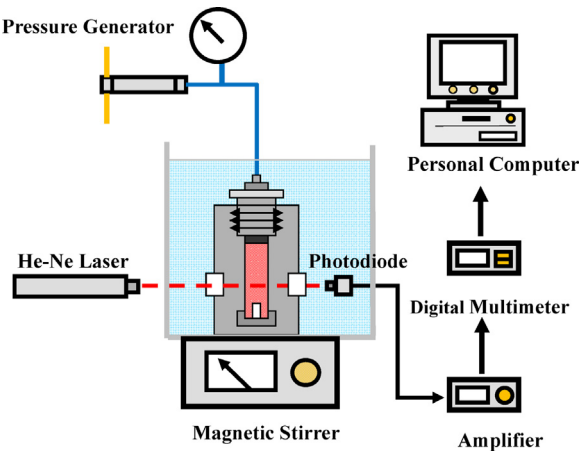

Fig. 1. Schematic diagram of high-pressure apparatus with a pair of sapphire windows. The transmitted laser beam passed through the erythrocyte suspension was detected by a silicon photodiode with an amplifier. The high-pressure vessel made of stainless steel was immersed in a temperature-controlled water bath.