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FUNCTIONAL CHARACTERISTICS OF CHEMICALLY MODIFIED HEMOGLOBIN DURING CIRCULATION IN THE BLOOD STREAM

E. P. Vyazova, M. A. Azhigirova, L. V. Fetisova, Yu. A. Litvinenko, I. R. Kolonina, and A. V. Sokolov

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Research into the production of an artificial oxygen carrier (AOC) based on chemically modified hemoglobin (Hb) has led to the obtaining of several compounds possessing oxygen-transport characteristics close to the properties of normal human blood, and capable of remaining for a long time in the blood stream [1, 6, 8]. The most widely used of these Hb derivatives is polyhemoglobin (PHb), which contains in its composition a covalently bound regulator of reversible oxygenation, namely pyridoxal-5-phosphate (PP) [5, 7], and which several workers regard as a potential AOC [4, 8]. One of the principal conditions for its suitability as an artificial substitute for erythrocytes, besides its long life in the blood stream, is the fact that its initially high functional activity remains unchanged during circulation, and it is this feature which determines its ability to maintain pO_2

Laboratory of Blood Protein Fractionation and Laboratory of Pathological Physiology, All-Union Hematologic Scientific Center, Ministry of Health of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR I. P. Ashmarin.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 111, No. 4, pp. 374-375, April, 1991. Original article submitted September 18, 1989.

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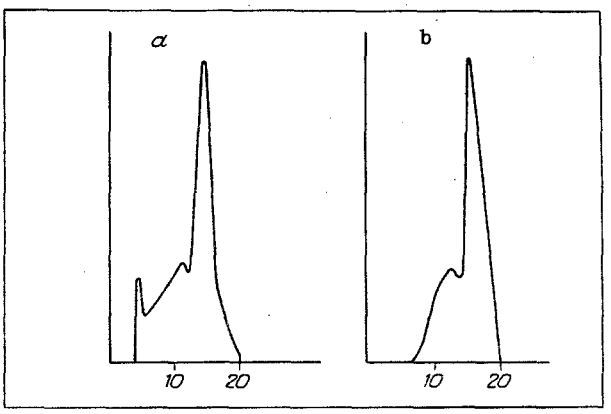

Fig. 1. Elution profiles of samples of chemically modified hemoglobins obtained by high-pressure liquid chromatography on TSK G 3000 SW 7.5 × 300 mm column. a) pHb-PP-1, b) pHb-PP-2. Abscissa, elution time (in min); ordinate, optical density at 408 nm. Rate of elution 1 ml/min, eluant 0.1 M phosphate buffer, pH 6.5.

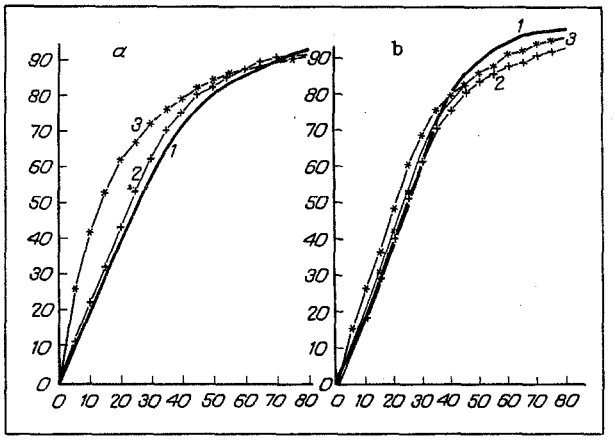

Fig. 2. Oxygen dissociation curves of chemically modified hemoglobins at different times of circulation in the blood stream: a) pHb-PP-1, b) pHb-PP-2. 1) Original substance, 2) after circulation for 4 h, 3) after circulation for 24 h. Abscissa, pO_2 (in mm Hg); ordinate, oxygen saturation of hemoglobin (in per cent).

in the tissues at a physiological level [9]. For this reason the aim of the investigation described below was to study the functional properties of a PHb-PP solution at various times after its use as replacement in acute blood loss in experimental animals, and also to establish correlation between these characteristics and the structure of the administered PHb-PP.

EXPERIMENTAL METHOD

Samples of PHb-PP, synthesized by ourselves and differing in their degree of polymerization, were used in the experiments [2].

The molecular-weight distribution of the PHb-PP specimens was studied by high-pressure liquid chromatography on TSK-250 columns ("Bio-Rad," USA); the relative percentages of the fractions with different molecular weights were determined by means of a 2220 integrator (LKB, Sweden).

Experiments were carried out on 17 mongrel male and female dogs weighing 15.2 ± 1.7 kg (nine dogs in Series PHb-PP-1 and eight dogs in Series PHb-PP-2), using a model of acute lethal blood loss (52 ± 3 ml/kg), under pentobarbital anesthesia. Blood was removed through the femoral artery, lowering the blood pressure to zero after which the lost blood was

replaced by 10% solutions of PHb-PP in a dose of 4 g/kg body weight. Blood samples were taken from the femoral artery immediately and 4 and 24 h after the end of infusion.

The HP concentration and methemoglobin (MT-Hb) concentration in the samples of blood plasma and solutions of PHb-PP were determined on a "CO-Oximeter 282" instrument (IL, USA).

Oxygen dissociation curves (ODC) in the test specimens were recorded on a "Hem-o-Scan" apparatus ("Aminco," USA) under physiological conditions: temperature 37°C, pH 7.4, pCO_2 40 mm Hg, 0.15 M NaCl. For quantitative analysis of the ODC values of P_{50} (partial pressure of oxygen at 50% saturation of Hb), characterizing affinity of PHb-PP for oxygen, Hill's coefficient, reflecting the degree of cooperativeness of the reversible oxygenation process, and A — the quantity of oxygen (in vols. %) given up by Hb during a drop of pO_2 from 90 to 40 mm Hg, similarly to what takes place in the body between an artery and vein, and calculated for Hb concentration in normal blood, were calculated.

EXPERIMENTAL RESULTS

Investigation of the degree of polymerization and of the molecular-weight distribution of the Hb derivatives used in the experiments showed that they differ significantly with respect to both these parameters (Fig. 1). PHb-PP-1 (Fig. 1a) contained about 6% of the fraction with mol. wt. of over 300,000 daltons and about 40% of the fraction from 300 to 100,000 daltons. In the composition of PHb-PP-2 (Fig. 1b) the high-molecular-weight fraction was absent, and the mean value was 25-30%. The fraction with mol. wt. of 70,000 daltons, corresponding to unpolymerized Hb, was about 54 and 70-75% for PHb-PP-1 and PHb-PP-2 respectively. As we showed previously [1, 3], this fraction is chemically modified Hb, predominantly with an intramolecular cross-linkage. Thus PHb-PP-2 differs from PHb-PP-1 not only in its significantly lower degree of polymerization, but also in its lower degree of dispersion by molecular weight. However, their functional characteristics were virtually identical: the value of P_{50} for the two substances was 26-29 mm Hg and Hill's coefficient 2.0-2.1.

The oxygen-transporting activity of the compounds was compared by analyzing pHb-PP in samples of blood plasma taken at different times after infusion. The study of the functional characteristics of pHb-PP-1 during circulation revealed considerable changes in the acid-transporting characteristics, as will be clear from Fig. 2a. Besides a successive fall in the values of P_{50}, down to 14 ± 1.5 mm Hg after circulation for 24 h, a change also was observed in the shape of the ODC, reflected in a lowering of the value of Hill's coefficient from 2.1 ± 0.5 to 1.6 ± 0.3. These changes invariably involved a decrease in the efficiency of oxygen transport: the calculated value of A fell from 4.2 to 2.2 vols. % after circulation for 24 h, only 52% of the original value. The observed decrease in functional efficiency of pHb-PP-1 correlates with data obtained by Moss et al. [8], and may be due to structural changes, which we established previously [2] and which are linked both with fractionation of pHb-PP during its isolation from the blood stream, and also with interaction with plasma proteins, taking place during circulation of the polyhemoglobin in the blood stream.

During the analogous experiments with pHb-PP-2 significantly smaller changes were observed in the original affinity for oxygen, and on that account the value of P_{50} after 24 h of observation had fallen only to 18.7 ± 0.5 mm Hg. Under these circumstances there was little change likewise in the value of Hill's coefficient, which was 1.9 ± 0.3, evidence of the virtual absence of any change in the shape of the ODC during the period of observation (Fig. 2b). In this case also A amounted to 90% of its original value (a decrease from 4.0 to 3.6 vols. %), which indicates only a slight decrease in the functional activity of pHb-PP-2.

The concentration of the test substances in the plasma 24 h after replacement of the lost blood was 3.3% for pHb-PP-1 and 3.1 ± 0.1% for pHb-PP-2. Oxidation of Hb, leading to the formation of functionally inactive MT-Hb, was more marked in the case of transfusion with pHb-PP-1: the MT-Hb concentration was 20.2-1.3% after circulation for 24 h, compared with 15.8 ± 1.0% for pHb-PP-2. Allowing for the above data on the calculated value of A it can be concluded that, with a virtually identical Hb concentration in the blood stream 29 h after infusion, the efficiency of oxygen transport by pHb-PP-2 was higher than by pHb-PP-1.

The experiments thus showed that correlation is observed between the degree of polymerization and heterogeneity of pHb-PP and the weakening of its functional activity in the course of circulation. Structural changes leading to changes in the ODC of Hb derivatives are evidently less marked during the circulation of pHb-PP with a narrower molecular-weight distribution in the blood stream. Consequently, during the creation of an AOC based on Hb, it is preferable to use compounds not containing high-molecular-weight (over 300,000 daltons) fractions, since these compounds can maintain the state of the body's oxygen transport more effectively in the late stages after infusion because of the better degree of preservation of their functional properties.

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THE Ca^{2+}-PUMP IN SMALL INTESTINAL MYOCYTES

V. K. Rybal'chenko

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KEY WORDS: plasma membrane; smooth-muscle cell; Ca^{2+}-pump; calmodulin; oxytocin

The total calcium content in smooth-muscle cells can be regarded as the resultant of three components: binding and release of the cation by chelating components of the cell and by subcellular structures, and exchange of Ca^{2+}-ions between the cell and the extracellular medium, realized through channel conductance and diffusion of ions along the electrochemical gradient, and also countergradient transport as a result of functioning of sodium-calcium exchange and the Ca-pump of the plasma membrane (PM). Particular attention is currently being paid to the latter, because establishment of the principles governing the functioning of the Ca-pump may provide the basis for the development of new approaches and methods of overcoming muscular function and correcting disturbances of motor activity in the digestive system.

This paper gives the results of experimental studies of the Ca-pump of PM of smooth-muscle cells and its regulation by calmodulin and oxytocin.

EXPERIMENTAL METHOD

The PM fraction was isolated from smooth-muscle tissue of the rabbit small intestine by differential centrifugation in a sucrose density gradient, and characterized with respect to marker enzymes and by electron microscopy; calcium accumulation by membrane vesicles was studied with the aid of a Ca-selective electrode and by the isotopic method [3].

EXPERIMENTAL RESULTS

The study of ATP-dependent accumulation of Ca^{2+} ions by vesicles of the PM fraction by means of a Ca-selective electrode showed that the membrane vesicles can accumulate the cation rapidly with an initial velocity of about 2 nmoles Ca^{2+}/mg protein/sec. Calcium accumulation by PM vesicles measured by the isotopic method after incubation for 30 min amounted to 20.0 ± 2.3 nmoles Ca^{2+}/mg protein, but in the presence of calmodulin (20-25 μg/ml) in the incubation medium, it rose to 27.5 ± 1.5 nmoles Ca^{2+}/mg protein (Figs. 1 and 2). Activation of the Ca-pump by calmodulin is dependent on concentra-

Laboratory of Membranology, Kiev University. (Presented by Academician of the Academy of Medical Sciences of the USSR S. E. Severin.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 111, No. 4, pp. 376-377, April, 1991. Original article submitted July 6, 1990.

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