A MODEL OF AN ARTIFICIAL OXYGEN CARRIER BASED ON POLYHEMOGLOBIN WITH FUNCTIONAL PROPERTIES CLOSE TO THOSE OF BLOOD

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The main problems facing the creation of an artificial oxygen carrier based on hemoglobin are how to increase its molecular weight in order to lengthen its circulation time in the blood stream [2, 9] and how to reduce the affinity of hemoglobin outside the erythrocyte for oxygen to values close to those of blood, in order to make the giving up of oxygen more efficient [8].

The first problem has been tackled by modifying hemoglobin with various high-molecular-weight compounds or by its polymerization [1, 4, 8, 9]. The affinity of these compounds for oxygen was identical with that of native extraerythrocytic hemoglobin [4], but higher than that of blood. The reason is the virtually complete absence of allosteric regulators of the reversible oxygenation process in solutions of hemoglobin and its derivatives [10].

The object of this investigation was to study a polyhemoglobin (PHb), synthesized by the writers, with pyridoxal-5'-phosphate (PP), a regulator of reversible oxygenation, attached to it chemically. The resulting compound (PHb-PP) possesses oxygen-dissociation characteristics and oxygen transport efficiency close to those of blood.

EXPERIMENTAL METHOD

Functional properties of the synthesized compounds were assessed by analysis of their oxygen dissociation curves (ODC). ODC were recorded under physiological conditions (pH 7.4, pCO_2 40 mm Hg, temperature 37°C, Cl^- concentration 0.15 M) on a Blood Gas Laboratory IL-217 apparatus.

The stability of PHb-PP was studied under physiological conditions at a partial pressure of oxygen corresponding to P_{50} of the substance, corresponding to the fastest formation of methemoglobin [5]. The test sample, with a Phb-PP concentration of 6%, was subjected to tonometry in Ringer's solution at 37°C, pCO_2 40 mm Hg, and pO_2 26-28 mm Hg.

The weighted mean molecular weight $\bar{M}_w$ was determined by the unstable equilibrium method on the Spinco E ultracentrifuge. The concentration gradient at the meniscus was calculated by linear extrapolation of the sedimentogram. That such extrapolation was possible was ensured by the experimental conditions [6]. The numerical mean molecular weight $\bar{M}_n$ was determined on a Knauer membrane osmometer, using double membranes from the same firm.

EXPERIMENTAL RESULTS

ODC of PHb-PP and of the original PHb, freed from chemically unbound phosphates by ultrafiltration, are given in Fig. 1. The value characterizing the position of the ODC on the graph and, consequently, the affinity of the test substances for oxygen, is the partial pressure of oxygen at 50% saturation of the measured solution (P_{50}). As Fig. 1 shows, attachment of PP to PHb led to a decrease in the affinity of the polymerized hemoglobin for oxygen, reflected in an increase in the value of P_{50} from 16-17 to 26-28 mm Hg, i.e., to characteristic values for human blood [7]. As a result of this, a solution of the new model of artificial

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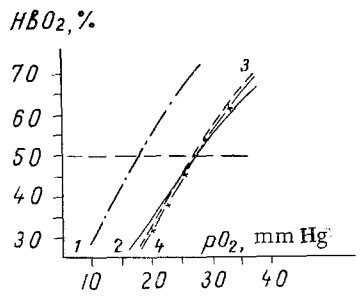

Fig. 1

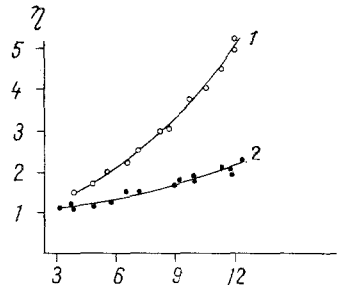

Fig. 2

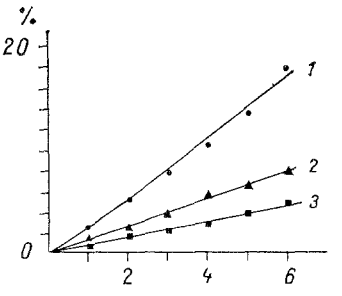

Fig. 3

Fig. 1. Oxygen dissociation curves: 1) 6% solution of PHb; 2) 6% solution of PHb-PP; 3) blood; 4) PHb-PP + blood.

Fig. 2. Dynamics of increase in methemoglobin: 1) 6% solution of PHb-PP; 2) 12% solution of PHb-PP + blood plasma; 3) 12% solution of PHb-PP + blood. Abscissa, time (in h); ordinate, methemoglobin concentration (in %).

Fig. 3. Relative viscosity as a function of concentration of PHb (1) and PHb-PP (2). Abscissa, hemoglobin concentration (in g/dl); ordinate, relative viscosity.

oxygen carrier based on hemoglobin acquires the property of giving up and combining with oxygen, in the presence of a physiological drop of partial oxygen pressures from 90 to 40 mm Hg, closely similar to that observed for blood. This overcomes one of the main objections to extraerythrocytic hemoglobin and its high-molecular-weight derivatives — the inefficient giving up of oxygen during a physiological drop in its partial pressures between arterial and venous blood.

To simulate the gas-transporting properties of PHb-PP solutions when used to compensate for lost blood, ODC of a mixture of equal volumes of blood and 12% PHb-PP solution, with the same oxygen capacity as blood, were studied. The results showed that the shape and position of the ODC of such a mixture were virtually the same as those of the ODC of blood, showing that a solution of PHb-PP has the same oxygen transporting capacity of blood (Fig. 1).

The stability of PHb-PP was studied by measuring the dynamics of its autooxidation. The increase in methemoglobin in the PHb-PP sample and also in mixtures of solutions of PHb-PP with plasma and blood (in equal volumes) are given in Fig. 2. The methemoglobin content in the PHb-PP solution after incubation for 7 h under the specified conditions was 17-18%. The rate of oxygenation of PHb-PP fell significantly in the presence of plasma — after 7 h the methemoglobin content in the sample was 7-9%. This can be explained by the presence of systems retarding the process of methemoglobin formation in blood plasma [5]. The increase in methemoglobin in a mixture of PHb-PP solution and blood in the course of 7 h was 4%, whereas on incubation of the original erythrocytes for the same length of time the accumulation of methemoglobin was 2-2.5%. The low total methemoglobin content in the mixture can be explained by the very slight degree of oxygenation of hemoglobin inside the erythrocytes.

It can be concluded from these results that the stability of PHb-PP solutions is sufficiently high in experiments conducted *in vitro*, simulating the conditions of blood loss.

The state of polydispersity of PHb-PP was assessed by determining the weighted mean and numerical mean molecular weights: The values obtained were $\bar{M}_w$ = 172,000 ± 16,000 and $\bar{M}_n$ = 120,000 ± 11,000. On the basis of these values the polydisperse width of the test model ($\bar{M}_w/\bar{M}_n$) was determined to be 1.4 ± 0.3. Comparison of values obtained previously for PHb [4], the polydisperse width of which is 2.8, shows that PHb-PP has a narrower molecular weight distribution than PHb not containing a regulator of oxygenation—deoxygenation.

The decrease in heterogeneity of molecular weight of PHb-PP compared with PHb made it desirable to study how the relative viscosity of this model depends on its concentration in solution. It was found that the narrower functional composition of PHb-PP has the result that, in equal concentrations, the relative viscosity of solutions of PHb-PP is appreciably lower than that of PHb (Fig. 3). It will be clear from Fig. 3 that in a concentration of 11-12 g/dl the relative viscosity of the PHb-PP solution was 2.0 whereas that of the PHb solution was 5.0. The concentration limit of PHb-PP solutions for injection into animals is

thus significantly higher. This is important not only in relation to increasing the oxygen capacity of the artificial oxygen carrier, but also in connection with the possibility of using various additional components during its administration, including polymers, in the attempt to develop a blood substitute of complex composition and polyfunctional action — an "artificial blood" [3].

A preliminary biological study of a solution of PHb-PP in experiments on noninbred albino mice and rats showed absence of toxicity in doses of 3 g/kg body weight, and an identical circulation time in the blood stream with that of PHb.

These investigations thus showed that the new model of an artificial oxygen carrier, developed by the writers, possesses basic functional properties similar to those of donors' blood.

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PEPTIDE MAPPING OF HUMAN ADENOVIRUS TYPE 6 AND SIMIAN ADENOVIRUS TYPE 7 HEXON AND CORE PROTEIN

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One of the most interesting objects in modern molecular biology and genetic engineering is the adenovirus, which is widespread in nature. By now about 80 serotypes of animal and human adenoviruses have been identified immunologically [5]. As criteria for isolation of subgroups of adenoviruses, their hemagglutinating properties, their oncogenicity, the homology of their nucleic acids as revealed by the hybridization method, and also the protein spectrum of the virions, determined electrophoretically, are usually used [6, 7, 11, 12].

In the investigation described below the method of tryptic mapping of proteins (fingerprinting) was used to determine similarity between human type 6 and Simian type 7 adenoviruses with respect to two major proteins — one external (hexon) and the other internal (core) [4].

EXPERIMENTAL METHOD

Adenovirus Ad6 was obtained in a culture of HeLa cells and SA7 virus in a culture of green guenon kidney cells [1, 2]. The viruses were extracted from infected cells by freezing and thawing, treated with freon-113, and purified by centrifugation in CsCl [10]. The

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