Chapter 2

2.1 Introduction

Several researchers developed artificial gills using oxygen carrier solution, which take up oxygen from water to air more efficiently compared with the membrane type artificial gills [1-3]. These artificial gills used the perfluorooctylbromide (PFOB) [1,2] or bovine red blood cell suspension (RBC) [3] as an oxygen carrier solution. Perfluorooctylbromide (PFOB) showed high oxygen solubility, large oxygen uptake rate from water to PFOB [1]. This oxygen carrier solution functioned oxygen storage tank for a sudden lack of oxygen in water. However, the oxygen release rate was significantly small compared with the oxygen uptake rate. The artificial gill using PFOB could not effectively transfer oxygen from water to air.

On the other hand, RBC suspension was used as a thermoresponsive oxygen carrier solution because it has thermoresponsive characteristics [3]. Bovine hemoglobin in RBC binds oxygen at lower temperatures (293 K) and it dissociates oxygen at higher temperatures (310 K). In oxygen uptake, the oxygen carrier solution was cooled to 293 K to increase oxygen affinity, and oxygen was taken up to oxygen carrier solution. On the contrary, in oxygen release, the oxygen carrier solution was heated to 310 K to decrease oxygen affinity, and oxygen was released to air. The oxygen transfer rate was increased considerably using this oxygen carrier solution. However, the device volume of the artificial gill using RBC is too large for practical use. Thus, efficient oxygen carrier solution is required to enhance oxygen transfer and reduce device volume. Furthermore, stability of an oxygen carrier solution should be improved for practical use.

In this chapter, an efficient oxygen carrier solution is developed with bovine
hemoglobin. The oxygen affinity of bovine hemoglobin was optimized by adding inositol hexaphosphate (IHP). The effective pH value and adding ratio of IHP were determined. An enzymatic methemoglobin reduction system was used to prolong the life of the oxygen carrier solution. The optimum range of temperature swing for an artificial gill was determined by considering the required flow rate of oxygen carrier solution and the required heat transfer rate.
2.1.1 Oxygen binding characteristics of Hemoglobin

In this chapter, an efficient oxygen carrier solution is developed with bovine hemoglobin. To develop more efficient oxygen carrier solution, the excess oxygen affinity must be controlled for the efficient transport of oxygen, and the oxygen-binding ability must be prolonged for the practical use. The characteristics of hemoglobin are important to control of oxygen affinity and prolong oxygen-binding ability.

2.1.1.2 Structure of hemoglobin molecule

Hemoglobin is a protein existing in red blood cells and works oxygen carrier in body. Hemoglobin is a spherical shaped heme-protein (6.4 x 5.5 x 5.0 nm) of which molecular weight is 64,500 (Fig. 2.1). Hemoglobin is consisted of four subunits, two α subunits and two β subunits. Each subunit is bonded with hydrophobic interactions, hydrogen and several ionic bonding. The aggregation of four subunits forms a cavity at center of hemoglobin molecule, which is termed “central cavity”. Each subunit has a heme which combines with oxygen, so that one hemoglobin molecule has four hemes. Heme is a plane porphyrin containing at a center. One side of the porphyrin combines with histidine (His F8) of globin chain. As shown in Fig.2.2, an oxygen molecule reversibly combines with the heme from the other side of porphyrin. The quaternary structure of hemoglobin greatly changes by combining with oxygen (from deoxyhemoglobin to oxyhemoglobin).
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Fig. 2.1 Structure of hemoglobin molecule [4-6]

Fig. 2.2 Structure of heme combining histidine [4-6]
2.1.1.3 Quaternary structure of hemoglobin molecule

Hemoglobin is consisted of two same protein chains, \( \alpha \) chain and \( \beta \) chain. Each \( \alpha \) chains contain 141 amino acid residues which form 7 \( \alpha \)-helixes. This formation is called globinfold. Each \( \beta \) chain contains 146 amino acid residues which form 8 \( \alpha \)-helixes. \( \alpha \) and \( \beta \) chains do not contain \( \beta \)-sheet. These protein chains contain hydrophobic amino acid residues which bond the same of other chains by hydrophobic interaction. The quaternary structure of hemoglobin is formed in this way. There are two contact surfaces between \( \alpha \) chain and \( \beta \) chain. The contact surface \( \alpha_1\beta_1 \) and \( \alpha_2\beta_2 \) are same structure. Similarly the contact surface \( \alpha_1\beta_2, \alpha_2\beta_1 \) are same structure. Though subunits are mainly assembled by hydrophobic interaction, hydrogen and several ionic bonding are 1/3 of maintaining energy of quaternary structure.

![Diagram of hemoglobin molecule]

Fig. 2.3 Contact surfaces of subunits [4-6]
There is difference of quaternary structure between oxyhemoglobin and deoxyhemoglobin. When oxyhemoglobin dissociates oxygen, contact surface $\alpha_1\beta_1$ ($\alpha_2\beta_2$) is not change, however, contact surface $\alpha_1\beta_2$ ($\alpha_2\beta_1$) is changes in the following way. Contact surface $\alpha_1\beta_2$ is intimately stabilized by hydrogen bonding. Oxyhemoglobin and deoxyhemoglobin use another amino acid residues for hydrogen bonding (Fig. 2.4). The change of amino acid residues for hydrogen bonding causes the change of quaternary structure of hemoglobin (Fig. 2.5).

**Fig. 2.4** Change of hydrogen bonding from deoxyhemoglobin to oxyhemoglobin [4]

**Fig. 2.5** Change of quaternary structure of hemoglobin [6]
There is a difference of ionic bonding among each subunit between oxyhemoglobin and deoxyhemoglobin. Four C-terminal groups of oxyhemoglobin subunits rotate freely. However, the terminal groups and the amino acid residues of deoxyhemoglobin subunits are fixed by ionic bonding (Fig. 2.6). Therefore, the structure of deoxyhemoglobin is more stabilized than oxyhemoglobin by several ionic bonding. Thus, quaternary structures of deoxyhemoglobin and oxyhemoglobin are called tense form and relaxed form, respectively.

Fig. 2.6 Ionic bonding among subunits of deoxyhemoglobin [5,6]
2.1.1.4 Cooperative oxygen binding of hemoglobin

An oxyhemoglobin dissociation curve has a peculiar sigmoid shape (Fig. 2.7). This indicates cooperative oxygen binding with hemoglobin, that is a binding of oxygen with a heme increases oxygen binding affinity of another heme in a hemoglobin molecules. The cooperative phenomenon is explained as follows. The Fe$^{2+}$ ion of deoxyhemoglobin is located in a position being 6 nm away from the plane of porphyrin toward the His F8. The Fe$^{2+}$ ion and His F8 are attracted to the plane of porphyrin by bonding of oxygen with heme (Fig. 2.8). The move of His F8 shifts the whole subunit and changes the quaternary structure. However, high energy is required to change the quaternary structure because the subunits are bonded each other by hydrophobic interaction, hydrogen and ionic bonding. Therefore, high oxygen partial pressure is required to combine first oxygen with heme. Once oxygen bonds with heme, oxygen combines easily with the other hemes, which results the cooperative phenomenon. Thus, half-saturated hemoglobin solution is a mixture of oxyhemoglobin and deoxyhemoglobin.
Fig. 2.7 Oxyhemoglobin dissociation curve [4-6]

Fig. 2.8 Move of His F8 by oxygen bonding [4,5]
2.1.2 Factors of controlling the oxygen affinity of hemoglobin

In living body, the oxygen affinity of hemoglobin is controlled to supply oxygen to the demand of peripheral tissues. It is controlled several condition, such as change in concentration of hydrogen ion, binding of allosteric effectors and change in temperature. These factors are described in this section.

2.1.2.1 Bohr effect

Oxyhemoglobin tends to dissociate oxygen with increasing hydrogen ion concentration. This phenomenon is called bohr effects, it is available for transport oxygen to peripheral tissues which are increased carbon dioxide concentration.

\[
\begin{align*}
    H_2O + CO_2 & \rightleftharpoons H_2CO_3 \\
    H_2CO_3 & \rightleftharpoons H^+ + HCO_3^-
\end{align*}
\]

Fig. 2.9 Equilibrium of CO₂ dissociation [4-6]

The principle of bohr effect is explained as follow. The quaternary structure of hemoglobin is changed by oxygenation, and pK of functional groups change because of change the periphery of them. Hence, pK of weak acid functional groups of deoxyhemoglobin is larger than that of oxyhemoglobin. Thus, deoxyhemoglobin is stronger base than oxyhemoglobin. As shown in Fig.2.10, Oxyhemoglobin and deoxyhemoglobin are equilibrated in water solutions. This equilibrium is shifted to left with increasing hydrogen ion concentration. Thus, oxygen affinity of hemoglobin is weakened with decreasing pH.
Fig. 2.10 Equilibrium of between oxyhemoglobin and deoxyhemoglobin [4-6]

Deoxyhemoglobin + 4O₂ ⇌ Oxyhemoglobin + nH₂

Fig. 2.11 Oxyhemoglobin dissociation curve at various pH values [4-6]
2.1.2.2 Allosteric effectors for hemoglobin

The oxygen affinity of hemoglobin in red blood cell is lower than that of naked hemoglobin. This is because the presence of 2,3-diphosphoglyceric acid (DPG) and it functions as a allosteric effector, which control the cooperative oxygen binding with hemoglobin. As shown in Fig.2.12, one molecule of DPG, which has strong negative charge, bonds to one deoxyhemoglobin at central cavity which has positive charge. DPG cross linkages amino acid residues of β subunits of hemoglobin and stabilized the quaternary structure of deoxyhemoglobin. Hence, high oxygen partial pressure is required to break the cross linkage, which result in the lower oxygen binding affinity. When an oxygen bonds with hemoglobin, the central cavity narrows and DPG is pushed out. Adenosine triphosphate (ATP), inositol hexaphosphate (IHP), pyridoxal complexed and band 3 protein exiting in red blood cell also affects to hemoglobin in the same manner as DPG (Fig.2.12, Fig.2.13). Table 2.2 summarizes binding constants to hemoglobin. Most of ATP exists as magnesium complex (ATP-Mg\(^{2+}\)) in red blood cell, which has lower binding constant. On the contrary, IHP combines with hemoglobin very strongly with six phosphates. It is used as an allosteric effector of a blood substitute [7-16]. This would be a efficient allosteric effector to control oxygen affinity of bovine hemoglobin, and useful for the development of oxygen carrier solution.
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(a) ATP  
(b) IHP  
(c) Pyridoxal complex

**Fig. 2.12** Binding site of DPG [4]  
**Fig. 2.13** Chemical structures of allosteric effectors [4]

**Fig. 2.14** Amino acid sequence of Band 3 [17]

**Table 2.1** Binding constants of several phosphate complexes to hemoglobin [4,6,17]

<table>
<thead>
<tr>
<th>Complex</th>
<th>Cellular concentration (mM)</th>
<th>Binding constant (L/mol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.3-DPG</td>
<td>7.0</td>
<td>$2.4 \times 10^4$</td>
</tr>
<tr>
<td>ATP</td>
<td>0.06</td>
<td>$1.2 \times 10^4$</td>
</tr>
<tr>
<td>ATP-Mg$^{2+}$</td>
<td>1.8</td>
<td>$8.3 \times 10^3$</td>
</tr>
<tr>
<td>ADP</td>
<td>0.2</td>
<td>$1.8 \times 10^3$</td>
</tr>
<tr>
<td>Band 3</td>
<td>0.024</td>
<td>$3.2 \times 10^3 \sim 1.1 \times 10^2$</td>
</tr>
<tr>
<td>Glucose-1,6-diphosphate</td>
<td>0.15</td>
<td>$1.0 \times 10^3$</td>
</tr>
</tbody>
</table>
2.1.2.3 Change in Oxygen affinity with temperature

The oxygen affinity of hemoglobin decreases with increasing temperature, and it increases with decreasing temperatures. This characteristic is used in artificial gill using red blood cell [3], oxygen separation membrane and blood oxygenation at low body temperatures. The dependence of oxygen affinity on temperature is explained as follow. The oxygen that binds to heme is bonded the surroundings of amino acid residues, such as histidine, by hydrogen bonding. The interaction between amino acid residues and oxygen are decreased with increasing temperature. Furthermore the interaction between oxygen and heme is also decreased with increasing temperatures.
2.1.3 Methemoglobin reduction system in red blood cell

In living body, methemoglobin, which is oxidized form of hemoglobin, reduced by methemoglobin reduction systems. It is available to prolong oxygen binding ability of hemoglobin in oxygen carrier solution. There are two patterns in methemoglobin reduction systems: one is the non-enzymatic reduction system, and the other is the enzymatic reduction system. As shown in Fig.2.15, non-enzymatic reduction is functioned with ascorbic acid and glutathione. This reduction system reduces small amount of total methemoglobin.

Enzymatic reduction has three patterns, it is functioned with NADH-cytochrome b<sub>5</sub> reduction enzyme, with NADH-dehydrogenase and NADH-flavin reduction enzyme. Methemoglobin reduction system with NADH-cytochrome b<sub>5</sub> reduction enzyme reduces 95% of total methemoglobin. In this chapter, this reduction system is used.

NADH is required to work the enzymatic reduction system. NADH is synthesized by Embden-Meyerhof-Parnas pathway (EMP pathway, Fig. 2.17). EMP pathway is started with glucose and ATP. However, ATP which is high-energy phosphate is tend to decompose, and is not synthesized again. Hence, ATP is produced with adding adenine and inosine, and EMP pathway is stared with produced ATP [18-20].
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Fig. 2.15 Methemoglobin reduction systems with ascorbic acid and glutathione [18-20]

Fig. 2.16 Methemoglobin reduction systems with NADH-cytochrome b5 reduction enzyme [18-20]

Fig. 2.17 EMP pathway [18]
2.2 Experimental

2.2.1 Preparation of the oxygen carrier solution

Bovine blood was centrifuged for 20 min at 2800 rpm with a centrifugal separator (H-103NN, Kokusan, Tokyo, Japan). The supernatant was removed and saline was added to the residual red blood cells at the same volume and centrifuged five times. The red blood cells were carefully added to a large quantity of pure water using a syringe to hemolyze the red blood cells as shown in Fig. 2.18. This solution was filtered using a plasma separator (Cascadeflo, Asahi Medical, Tokyo, Japan) and sterilized using a sterile filtration membrane (Milllex-GP<sub>50</sub>, pore size: 0.22 µm, Millipore, Bedford, MA). Then a stroma free hemoglobin solution was prepared. The stroma free hemoglobin solution was concentrated to 5.43 µmol/cm<sup>3</sup> using a high-performance dialyzer (AM-SD-150H, Asahi Medical) (Fig.2.19). The hemoglobin concentration was measured by the hemoglobin cyanidation method [24,25] using Hemoglobin-test-Wako (Wako, Osaka, Japan). Inositol hexaphosphate dipotassium salt (IHP, Sigma, St.Louis, MO) was added to the concentrated hemoglobin solution at designated ratios with respect to the hemoglobin concentration. D-glucose (Wako), adenine (Wako), β-NAD<sup>+</sup> (Wako) and inosine (Sigma) were added to the hemoglobin solution at molar ratios of hemoglobin to D-glucose to adenine to β-NAD<sup>+</sup> to inosine of 5.7 : 100 : 2.0 : 1.0 : 5.0, to serve as the methemoglobin reduction system. pH was adjusted using citrate-phosphate buffer, and an oxygen carrier solution was prepared.
Fig. 2.18 Schematic of hemolysis of bovine red blood cells

Fig. 2.19 Schematic of apparatus for separating stroma and concentration of hemoglobin
2.2.2 Measurement of oxyhemoglobin dissociation curve of the oxygen carrier solution

The oxyhemoglobin curve which represents oxygen affinity of hemoglobin was measured with the apparatus shown in Fig. 2.20. The oxygen carrier solution diluted to 0.140 \(\mu\text{mol/cm}^3\) was placed in a sealed flask and oxygen or nitrogen gas was blown into the solution. The solution was thoroughly stirred and oxygen partial pressure was measured using a dissolved oxygen meter (TOX-90i, Toko Chemical Laboratories, Tokyo, Japan). Then 10 cm\(^3\) of the solution was sampled and applied to a flow cell type spectrophotometer (UV-1200, Shimadzu, Kyoto, Japan). Absorbances at 554 and 576 nm were measured for deoxy and oxyhemoglobin peaks, respectively, with an isosbestic point at 584 nm to determine the degree of oxygen saturation of hemoglobin. The relationship between oxygen saturation \(S(-)\) and oxygen partial pressure \(p_c\) (Pa) was represented by Hill’s equation [4]

\[
S = \frac{Hp_c^n}{1 + Hp_c^n} \quad (2.1)
\]

where \(n\) (-) and \(H\) (Pa\(^n\)) are Hill constants.
2.2.3 Measurement of oxygen transfer rate through the oxygen carrier solution

The oxygen transfer rate through the oxygen carrier solution was measured using a miniaturized hollow fiber membrane module. The structure of hollow fiber module is shown in Fig.2.21. The hollow fiber was made of 0.2 μm-silicone-coated porous polypropylene [26,27] with an inner diameter of 0.25 mm, an outside diameter of 0.3 mm and a membrane thickness of 25 μm. The number of hollow fibers, effective length of the hollow fiber and membrane surface area were 200, 100 mm and 0.0188 m², respectively.
The experiments were carried out in two systems, consisting of uptake of oxygen from water to the oxygen carrier solution (water-carrier system) and its release it from the carrier to air (carrier-air system). Fig.2.22 shows the experimental circuit of the water-carrier system.

The oxygen carrier solution was caused to flow inside the hollow fibers at a flow rate of 0.0833 cm$^3$/s by a syringe pump. Air-saturated water flowed outside the hollow fibers at a flow rate of 1.33 cm$^3$/s by gravity. Temperature was maintained at 293K. The oxygen partial pressures of the oxygen carrier solution and water were measured using dissolved oxygen meters (TOX-90i) placed at the inlet and outlet of the membrane module. The oxygen transfer rate $N$ (mol/s) was obtained at both oxygen carrier and water sides, as

$$N_W = Q_W \{ \alpha (p_{\text{Win}} - p_{\text{Wout}}) \}$$

$$N_C = Q_C \{ \beta_C (S_{\text{out}} - S_{\text{in}}) + \alpha (p_{\text{Cout}} - p_{\text{Cin}}) \} \quad (2.2)$$

$$N_C = Q_C \{ \beta_C (S_{\text{out}} - S_{\text{in}}) + \alpha (p_{\text{Cout}} - p_{\text{Cin}}) \} \quad (2.3)$$

where $Q$ (cm$^3$/s) is the flow rate, $\alpha$ (mol /(cm$^3$·Pa)) the physical solubility of oxygen, $p$ (Pa) the oxygen partial pressure, $\beta_C$ (mol/ cm$^3$) the amount of oxygen chemically bonded with a unit volume of the oxygen carrier solution, and $S$ (−)
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Fig. 2.22 Schematic of apparatus for measuring oxygen uptake rate from water to oxygen carrier solution

Fig. 2.23 Schematic of apparatus for measuring oxygen release rate from oxygen carrier solution to expired air
the degree of oxygen saturation of hemoglobin. Subscript \( W \) is water, \( C \) the oxygen carrier solution, and in and out indicate the module inlet and outlet, respectively.

Fig. 2.23 shows the experimental circuit of the carrier-air system. The flow rate of oxygen carrier solution was caused to flow outside the hollow fibers at a flow rate of 0.333 cm\(^3\)/s by a syringe pump. Expired air (oxygen partial pressure: 15.5 kPa) flowed inside the hollow fibers at a flow rate of 0.167 cm\(^3\)/s. Temperature was set to 298 K, 303 K or 310 K.

The oxygen transfer rate \( N \) (mol/s) was obtained at both the oxygen carrier and air sides as

\[
N_c = Q_c \left( \beta_c (S_{cin} - S_{cout}) + \alpha (p_{cin} - p_{cout}) \right) \tag{2.4}
\]

\[
N_A = Q_A \left( \frac{p_{Aout} - p_{Ain}}{p_0} \right) \tag{2.5}
\]

where \( p_0 \) (Pa) is the total pressure of air, subscript \( A \) is the air. The heat transfer rate \( q \) (J/s) required to heat the oxygen carrier solution was obtained by

\[
q = Q_c C_p \rho_c \Delta T \tag{2.6}
\]

Where \( \Delta T \) (K) is the range of temperature swing of the oxygen carrier solution, \( C_p \) (2.88 J/(g·K)) the specific heat capacity, \( \rho_c \) (1.08 g/cm\(^3\)) the density, and \( Q_c \) (ml/min) the flow rate of the oxygen carrier solution.
2.3 Results and Discussion

2.3.1 Optimization of oxygen affinity of the oxygen carrier solution

Fig. 2.24 shows the oxyhemoglobin dissociation curve of the oxygen carrier solution at various ratios of IHP to hemoglobin concentration (IHP:Hb). pH was adjusted to 7.4 and temperature was 293 K and 310 K. The ratio of IHP to hemoglobin ranged from 0:1 (stroma free hemoglobin) to 10:1. The oxyhemoglobin dissociation curve shifted to the right with increased ratio of IHP to hemoglobin. However, the dissociation curves showed the same curve when the ratios were 5:1 and 10:1 at 293 K. This indicates that the oxygen affinity of the hemoglobin solution did not change with an increase in the ratio of IHP to higher than 5:1. In consideration of the oxygen partial pressure for the practical use of an artificial gill, the optimum ratio of IHP to hemoglobin was taken as 5:1.
Fig. 2.24 Oxyhemoglobin dissociation curve of the oxygen carrier solution at various ratios of IHP to hemoglobin concentration (IHP:Hb): (a) 293 K, (b) 310 K

Fig. 2.25 shows the oxyhemoglobin dissociation curves at various pH value. The ratio of IHP to hemoglobin was set at 5:1. The oxyhemoglobin dissociation curve shifted to the right with decreased pH. The dissociation curves at pH of 6.9 and 6.5 were similar. Because lower pH promoted proton oxidation of hemoglobin, the optimum pH was 6.9.

Fig. 2.26 shows the oxyhemoglobin dissociation curves at various temperatures. The ratio of IHP to hemoglobin and pH were set at 5:1 and 6.9, respectively. Temperature was set at 293 K, 298 K, 303 K or 310 K. The oxyhemoglobin dissociation curve shifted to the right with increased temperature. The degree of shift per width of temperature swing was high at higher temperatures. Equilibrium between oxyhemoglobin and deoxyhemoglobin shifted toward deoxyhemoglobin with increases in the temperature. Because IHP bonds only to deoxyhemoglobin, IHP functioned more effectively at higher temperatures.
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**Fig. 2.25** Oxyhemoglobin dissociation curve of the oxygen carrier solution at various pH values (IHP:Hb=5:1, 310 K)

**Fig. 2.26** Oxyhemoglobin dissociation curve of the oxygen carrier solution at various temperatures (IHP:Hb=5:1, pH 6.9)
2.3.2 Stability of hemoglobin solution

Fig. 2.27 shows the absorption spectra of hemoglobin with and without the methemoglobin reduction reagents. A higher absorption spectrum was observed at lower wavelengths when the methemoglobin reduction reagents were absent, indicating the presence of methemoglobin. A higher absorption spectrum was not observed with the methemoglobin reduction system. The amount of oxygen chemically bonded to a unit volume of hemoglobin solution $\beta_C$ was obtained by the mass balance of oxygen represented by equations (2) and (3). The value was 18.0 $\mu$mol/cm$^3$, while the value of the concentrated hemoglobin solution (5.43 $\mu$mol/cm$^3$) was 21.7 $\mu$mol/cm$^3$. This demonstrates that 82.9% of hemoglobin participated in oxygen bonding.

Fig. 2.27 Absorption spectra of hemoglobin with and without the methemoglobin reduction reagents
2.3.3 Oxygen transfer rate through concentrated hemoglobin solution

The oxygen transfer rate from water to the oxygen carrier solution (the oxygen uptake rate) was measured. Fig. 2.28 shows the dependence of the oxygen uptake rate from water $N_W$ on oxygen partial pressure of the oxygen carrier solution at module inlet $p_{Cin}$ of the water-carrier system. The oxygen transfer rate markedly increased at lower partial pressures, because oxygen partial pressure differences between the oxygen carrier and water are high, and the oxyhemoglobin dissociation sharply rises at these partial pressures as shown in Fig.2.9. These enhanced the oxygen transfer rate from water.

![Graph showing oxygen uptake rate vs oxygen partial pressure](image)

**Fig. 2.28** Dependence of the oxygen uptake rate from water on oxygen partial pressure in the oxygen carrier solution at module inlet
The oxygen transfer rate from the oxygen carrier solution to air (the oxygen release rate) was measured. Fig. 2.29 shows the dependence of the oxygen release rate to expired air on oxygen partial pressure of the oxygen carrier solution at module inlet $p_{Cin}$ of the carrier-air system. Oxygen release rates increased with temperature. The increase of oxygen release rate at 310 K decreased at higher oxygen partial pressures because slope of the oxyhemoglobin dissociation curve was gentle at higher partial pressures.

![Fig. 2.29](image)

**Fig. 2.29** Dependence of the oxygen release rate to air on oxygen partial pressure in the oxygen carrier solution at module inlet

The flow rate of the oxygen carrier solution required to supply 0.223 $\mu$mol /s oxygen, which is required for human respiration at rest and the heat transfer rate required to heat the oxygen carrier solution were calculated. Oxygen partial pressure of the oxygen carrier solution at the module outlet $p_{Cout}$ of the water-carrier system (293 K) was set at 15.7 kPa. As shown in Fig.2.30, the
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oxygen partial pressures at the module inlet $p_{Cin}$ of the carrier-air system after the temperature swings were 19.5 kPa, 24.9 kPa and 44.3 kPa for temperature swings to 298 K, 303 K and 310 K, respectively.

Oxygen transfer rates under these conditions were calculated from experimental data. Fig.2.31 shows the flow rate of the oxygen carrier solution required to supply 0.223 µmol/s oxygen and the required heat transfer rate. The required flow rate of oxygen carrier solution markedly decreased and the heat transfer rate was relatively low when the temperature was changed from 293 K to 310 K. Therefore, the optimum range of temperature swing of the oxygen carrier solution was from 293 K to 310 K. The required carrier flow rate and heat transfer rate at this condition were 0.362 dm³/s and 19.4 kJ/s, respectively, which greatly reduced device volume and energy for running artificial gill. Optimization of module structure will further enhance oxygen transfer rate, which enables to make a compact and portable artificial gill.

This condition saves much energy of artificial gill device. Improved high performance module for oxygen transfer will enhance oxygen transfer, increasing the efficiency of artificial gill device.
Fig. 2.30 Oxygen partial pressure in oxygen carrier solution at oxygen release module inlet after temperature swing

Fig. 2.31 Flow rate of oxygen carrier solution and required heat transfer rate
2.4 Conclusions

An efficient oxygen carrier solution was developed with bovine hemoglobin. The oxygen affinity of hemoglobin is adjusted by inositole hexaphosphate and pH. The oxygen transfer rate is markedly increased by changing the temperature from 293 K to 310 K at an optimized ratio of inositole hexaphosphate to hemoglobin of 5:1 and a pH of 6.9. The oxygen binding ability is prolonged with the enzymatic methemoglobin reduction system. The required flow rate of the oxygen carrier solution is markedly decreased by setting these conditions and the heat transfer rate is relatively low which enables to make a compact and efficient artificial gill.
Chapter 2

List of symbols

\begin{itemize}
\item[$A$] Membrane surface area \hspace{1cm} (m$^2$)
\item[$C_p$] Specific heat capacity of the oxygen carrier solution \hspace{1cm} (2.88 J/(g·K))
\item[$H$] Hill constant \hspace{1cm} (Pa·m$^{-n}$)
\item[$N$] Oxygen transfer rate \hspace{1cm} (mol/s)
\item[$n$] Hill constant \hspace{1cm} (-)
\item[$p_c$] Oxygen partial pressure of oxygen carrier solution \hspace{1cm} (Pa)
\item[$Q$] Flow rate \hspace{1cm} (mol/s)
\item[$q$] Heat transfer rate \hspace{1cm} (J/s)
\item[$S$] Degree of oxygen saturation of hemoglobin \hspace{1cm} (-)
\item[$\alpha$] Physical solubility of oxygen \hspace{1cm} (mol/(m$^3$ Pa))
\item[$\beta_c$] Amount of oxygen chemically bonded with a unit volume of oxygen carrier solution \hspace{1cm} (mol/m$^3$)
\item[$\Delta p$] Logarithmic mean difference of oxygen partial pressure \hspace{1cm} (Pa)
\item[$\Delta T$] Range of temperature swing of oxygen carrier solution \hspace{1cm} (K)
\item[$\rho_c$] Density of the oxygen carrier \hspace{1cm} (1.08 g/cm$^3$)
\end{itemize}
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Subscripts

\[ A \quad \text{Air} \]
\[ C \quad \text{Oxygen carrier solution} \]
\[ \text{in} \quad \text{Module inlet} \]
\[ \text{out} \quad \text{Module outlet} \]
\[ W \quad \text{Water} \]
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