The study of carbon monoxide placenta blood hemoglobin oxygenation

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Abstract. With the great progress of hemoglobin-based blood substitutes,however, theyallhave a same problem that is hard to get effective stable structure of hemoglobin.The hemoglobin can be oxidized into met hemoglobinin the process of purification,lead to the loss of the function of carrying and transporting oxygen. The combination of CO and placenta blood hemoglobin ability is far higher than that of O2, the structure and properties of carbon monoxide hemoglobin (HbCO) has more stable.Red blood cell (RBC)is mixed with organic solvent for hemolysis and centrifuged for removal of stroma.To prevent MetHb formation during the procedure, Hb was carbonylated in advance.Excessive carbon monoxide pumped to hemoglobin,hemoglobin is converted intoHbCO. HbCO is decarbonylated to regenerate HbO2 by exposing the solution to oxygen and visible light, the content of HbCOand HbCOis measured by blood gas analyzer.By comparing the liquid membrane and film boiling method, determination of oxygenation results.Measured liquid membrane oxygenation ismore effective than hollow fiber module method.Through the liquid membrane method, large quantities of hemoglobin can be treated in production,it also can reduce the loss of hemoglobin during purification,increase oxygen affinity.

1. Introduction

The development of hemoglobin-based blood substitutes has now advanced to the stage that several products are being tested in clinical trial [1-2]. These studies, as well as numerous animal studies, have demonstrated that the administration of relatively large quantities of extracellular hemoglobin or hemoglobin derivatives may lead to a variety of undesirable toxic side effects [3].It has been known for more than a decade that the administration of native hemoglobin causes severenephrotoxicity's, which are apparently associated with the excretion of hemoglobin dimers via the kidneys.Initially,it was suggested that at least some of these toxic effects may be due to impurities that remained present in the various hemoglobin preparations, such as endotoxins and/or stromal phospholipids [4-6].It is proposed that this limitation can be overcome byincreasing molecular size and oxygen affinity.We have established a new method by taking the advantage of excellent stability of carbonylhernoglobin (HbCO), then remove CO from Hb before purification.

2. Materials and methods

Preparation of stroma-free HbCO

Washing hemoglobin:RBCs were obtained from Tianjin union stem cell gene engineering company. The placenta blood was put into centrifugal tube, Volume should not exceed 1/3 of centrifuge tube,added the physiological saline to two-thirds of the centrifugal tube, centrifuged by high-speed centrifuge table (≥2,800g,≤15min,4℃).Thesupernatant was removed after the end of centrifugation, repeat the process 3 to 5 times until the supernatant was close to colorless.

Carbonylation: The washed blood was transferred to the conical flask, added an equal volume of saline to dilute. The CO was pumped into the bloodstream by syringe needle, the time was about 30 min, and the whole process would find that the color of the blood changed from red to dull-red.The solution was centrifuged by high-speed centrifuge table (≥2,800g,≤15min,4℃),the supernatan was removed after the end of centrifugation.

Membrane remove: first, the centrifugal solution (RBC) was put into the ice water to cool to 4℃, then, RBC was mixed with CH_2Cl_2 for hemolysis, the volume of RBC and CH2Cl2 is 1:0.2. The mixed solution was shaken for 3 min and then centrifuged (2 8,000g, 15 min). An HbCO solution was separated as a top layer. After repeating the procedure again, residual CH_2CI_2, in the HbCO solution was removed out at ca. 20 torr at 40℃ in dark.

The oxygen of HbCO

Method 1:hollow fiber module method

COHb solution was frown through hollow fiber module, with the condition of light, ice water bath, oxygen, CO was removed from HbCO solution, it is purposed to stabilize hemoglobin.

Scheme 1:Blood goes around hollow fiber, the oxygen goes into the hollow fiber.

20 ml $NaHCO_3$ was added into the 40 ml removed membrane solution to accommodate pH. The pH of HbCO was 8.25. According to Bohr Effect: with the loss of the pH value, the oxygen affinity of hemoglobin was receded. So control pH of HbCO fluid for weak alkaline. The test was started in a bottle,the content of HbCO and HbCO is measured by blood gas analyzer.

Scheme 2: Change the way of oxygen and blood circulation mode, other conditions are not changed. The oxygen goes around hollow fiber, blood goes into the hollow fiber.

Method 2:liquid membrane method

To make HbCO solution forming a very large area of liquid membrane on the surface, the gas exchange area of HbCO was increased, it is more favorable for CO and O2 exchange.

Scheme 1: pumping in oxygen and evacuated replacement

$NaHCO_3$ was added into the 80 ml removed membrane solution to accommodate pH. The pH of HbCO was 7.8. The HbCO solution was transferred into an eggplant shaped bottle after pH adjustment, connected to the rotary evaporation apparatus for oxygenation in the condition of light and ice water bath. the whole process of experiment was need to access to oxygen, need a vacuum after 10 min oxygen flux, continuously repeating the experiment operation, every once in a while after taking 3 ml HbCO solution, diluted with 20 ml distilled water, with 0.22μm membrane filter. The content of HbCO and HbO_2were measured by blood gas analyzer.

Scheme 2: In combination with oxygen and evacuated replacement

Phytic acid 0.76 g and 0.76 g ascorbic acid were dissolved in 40 ml distilled water, adjusted the pH to 7.5, mixing with 40 ml removed membrane solution. In the 80 ml mixed solution, the final concentration of phytic acid was10 mmol/L, the final concentration of ascorbic acid was 5 mmol/L. The mixed solution was transferred into an eggplant shaped bottle, connected to the rotary evaporation apparatus, and only used its rotation function for oxygenation with the condition of light and ice water bath. At the beginning of the experiment , the mixed solution was, then pumped in oxygen 10 min, vacuumized 10 min. Continuously repeating the experiment operation, every once in a while after taking 3 ml HbCO solution, diluted with 20 ml distilled water, with 0.22μm membrane filter. The content of HbCO and HbO_2 were measured by blood gas analyzer.

3. Results and analysis

3. 1 The result of hollow fiber module method

The result of scheme 1:

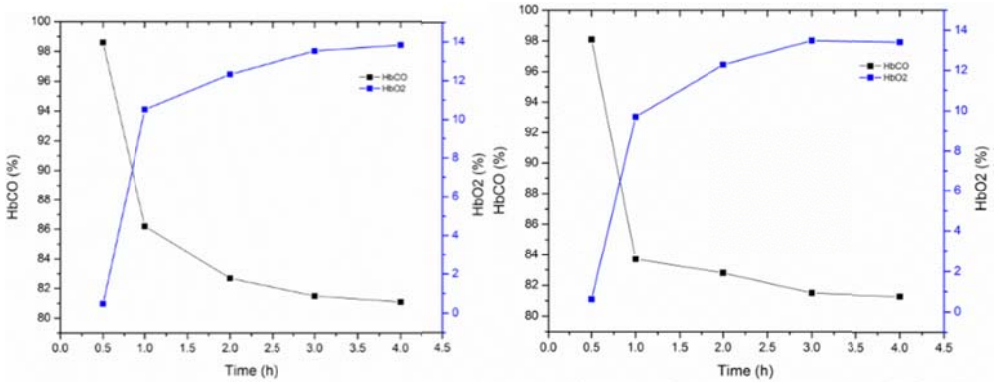

Fig.1. Speed of transformation from HbCO to HbO_2 Fig.2. Speed of transformation from HbCO to HbO_2

At the beginning of reaction,the percentage of HbCO fell sharply, as the experiment progresses, the speed decreases, three hours later, thepercentage was no longer droped, eventually remained at around 82%, and the percentage ofHbO_2 increased slowly.

The result of scheme 2:

Though the way of oxygen and blood circulation mode had beenchanged, the HbCOsolution can contact with more sufficient oxygen, but on the whole the results did not have a material impact, the percentage of HbCO still no longerdroped after 81%.

By comparing the results of two schemes, the process of experiment was taken by two different ways, but got the same results. Two experimental schemes finally measured the percentage of HbCOwas around 81%, cannot be further reduced. Method ofhollow fiber module does not apply to HbCOoxygenation.

3. 2. Theresult of liquid membrane method

The result of scheme 1:

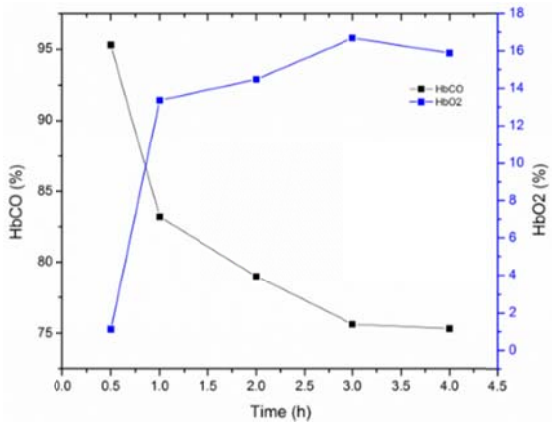

Fig.3. Speed of transformation from HbCO to HbO_2

It had no longer a significant change untilthe percentage ofdropped to about 78%. By figure 3 wefound that the percentage of HbCO fell fast within the starting 2h, but after reaction for 2 h, the percentage of HbCOkept balance, there had no reduction.

The result of scheme 2:

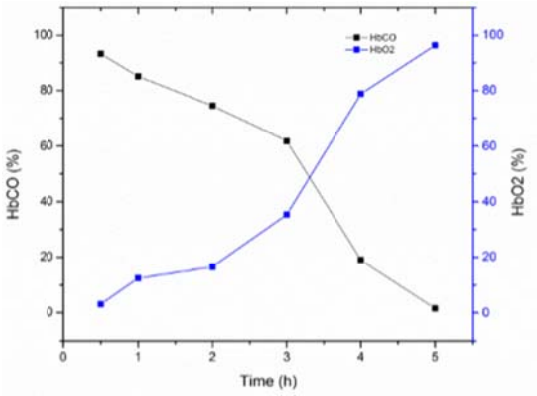

Fig.4. Speed of transformation from HbCO to HbO_2

Aswe could see from figure 4, the percentage of HbCO dropped to 1.5%, HbO2 in quantity from 3.1 % to 96.4%, and the percentage ofincreased quickly, HbCO wasconverted to HbO_2within 5 h. The metHb was 3.1%.

By comparing the results of two schemes, we found thatthe phytic acidwas of a similar effect with2, 3 – DPG, it can combineHband promote the release of oxygen. Vc wasa strong reducibility, Hbcould not been oxidized to high iron statusduring experiment process.

4. Conclusion

With the result of two methods, there had a conclusion that liquid membrane method (in combination with oxygen and evacuated replacement) was more effective than hollow fiber module method. Hb could keep more stable and increase oxygen affinity by using liquid membrane method in oxygenation experiment. It has a significant preparation for Hb purification.

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