

## ALPHA-ALPHA CROSS-LINKED HEMOGLOBINS

## BACKGROUND OF THE INVENTION

This invention relates to a modified hemoglobin composition, used as a blood substitute and a blood plasma expander. There is a critical need within the medical industry for blood substitutes and blood volume expanders. This need occurs not only because of the shortage of donor blood in bloodbanks, but also because of many problems that commonly exist with donor bloodbank practices. For example, there is an increasingly significant risk of disease transmission such as acquired immunodeficiency syndrome, commonly referred to as "AIDS", and even much more commonly, a real hepatitis risk. The shelf life of whole blood is also relatively short, not usually lasting longer than 30 days. There is also the problem of the need for blood typing, etc. with donated whole blood samples.

Accordingly, there is a very real and continuing need which has existed for some time, for blood substitutes or blood plasma expanders which can be conveniently prepared from a base hemoglobin source, such as discarded blood samples. This invention has as its primary objective, the fulfillment of this continuing need.

Currently there are two available possible routes for blood substitutes and blood plasma expanders which are being investigated. The first is fluorocarbons and the second is modified hemoglobins. The modified polyhemoglobins are represented by U.S. Pat. No. 4,001,401. Fluorocarbons are also receiving much active investigation at the present. However, it is believed unlikely that fluorocarbons will ever successfully take over the market for blood substitutes or blood plasma expanders because these are known to at times block the natural immune system. In addition, the use of fluorocarbons is limited to situations in which high partial pressures of oxygen can be administered. They do not have a sufficiently high oxygen binding capacity for use under normal environmental conditions. Thus, while currently available materials do represent a contribution and some advancement in medical sciences directed towards the concept of a blood substitute and blood plasma expander, there is currently nothing of significant commercial affect available on the market.

There is also the problem of not only developing an effective oxygen carrying blood substitute which will effectively release the oxygen for body use, but also developing a composition which will not be renally eliminated. A natural mammalian hemoglobin is a tetramer, which in plasma will in the oxy form have a tendency to split into two alpha-beta dimers, each having a molecular weight of approximately 32,000. These dimers are small enough to be filtered by the kidneys and be excreted, with the result being a potential for renal injury and a substantially decreased intravascular retention time.

It therefore becomes readily apparent that there is a continuing need for a therapeutic product useful as a blood substitute and blood plasma expander, which will effectively bind oxygen, but not bind it so tightly that it will *not* be released for body use; and, for development of a product which will not split into alpha-beta dimers, capable of rapid elimination by the renal route as well as loss from the circulation through capillary beds in other tissues.

Accordingly, another primary object of the present invention is to prepare an effective blood substitute and blood plasma expander from modified hemoglobin.

Another objective of the present invention is to prepare a blood substitute and blood plasma expander based on a derivative of hemoglobin cross-linked specifically between the alpha chains.

Yet another object of the present invention is to prepare an effective modified hemoglobin which has a relatively low oxygen affinity, that is, will release the oxygen easily for body use, but which at the same time is incapable of being split into alpha-beta dimers and as a result, rapid renal elimination is prevented.

Yet another objective of the present invention is to prepare a modified hemoglobin, cross-linked between the alpha chains at specifically Lys 99 Alpha<sub>1</sub> to Lys 99 Alpha<sub>2</sub>.

Yet another objective of the present invention is to prepare an even further modified hemoglobin, which is not only alpha-alpha cross-linked but one which is also selectively modified with an acylating agent at the 2,3-diphosphoglycerate binding site, located between the beta chains, to introduce a negatively charged group within this region which even further enhances hemoglobin oxygen release, making the cross-linked composition even more effective in certain applications. Such derivatives having a markedly reduced oxygen affinity may be particularly useful, for example, in the treatment of ischemia (i.e., heart attacks and strokes) as well as in the replacement of blood loss.

A still further objective of the invention is to provide a blood substitute and plasma expander that is readily available, stable under prolonged storage, and which can be used without significant disease transmission risk.

A yet further objective of the present invention is to provide an alpha-alpha cross-linked modified hemoglobin having a molecular weight of approximately 64,000, which will not split into dimers of about 32,000 molecular weight, during use.

A still further objective of the present invention is to provide a cross-linked hemoglobin, which is alpha-alpha cross-linked, and substantially free of hemoglobins modified at other sites, thus diminishing the risk of antigenic reaction which can occur with random modifications of hemoglobin.

## BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 is an elution profile monitored by the optical density at 540 nm for the purification of the alpha-alpha cross-linked derivative by chromatography on a DEAE (diethylaminoethyl) cellulose column.

FIG. 2 is an oxygen equilibrium curve showing the fraction of oxygen bound as a function of the log of the partial pressure of oxygen, for both normal adult hemoglobin (closed circles) and the alpha-alpha cross-linked derivative (Lys 99 Alpha<sub>1</sub>-Lys 99 Alpha<sub>2</sub>) (open circles).

FIG. 3 is the difference electron density contour map between the alpha-alpha cross-linked derivative and native deoxyhemoglobin superimposed upon the atomic model of hemoglobin in the region of the cross-link.

## SUMMARY OF THE INVENTION

A new hemoglobin composition, which is intramolecularly cross-linked between Lys 99 Alpha<sub>1</sub> and Lys 99 Alpha<sub>2</sub>, and blood substitutes and blood plasma expanders comprising a therapeutically effective amount of