

THERAPIES USING HEMOPROTEINS

RELATED APPLICATIONS

This application is a continuation of International Appli- 5
cation No. PCT/US99/15487, which designated the United
States and was filed on Jul. 9, 1999, published in English,
which claims the benefit of U.S. Provisional Application No.
60/092,372, filed on Jul. 10, 1998. The entire teachings of
the above application are incorporated herein by reference. 10

GOVERNMENT SUPPORT

The invention was supported, in whole or in part, by a 15
grants HL52529 and HL59130 from National Institutes of
Health. The Government has certain rights in the invention.

BACKGROUND OF THE INVENTION

Hemoproteins are a group of proteins which contain a 20
heme prosthetic group. They include cytochromes and
hemoglobins. These proteins play a critical role in the
bioenergetics of aerobic metabolism in mammals, and indi-
viduals with mutations or deficiencies in these proteins can
have deficiencies in oxygen delivery (e.g., sickle cell ane- 25
mia) and electron transport (e.g., Leigh syndrome) which
are associated with high rates of mortality. Indeed, the impor-
tance of balanced oxygen delivery and consumption to
ensure normal metabolism (e.g., oxidative phosphorylation)
and avoid oxidative stress is well established. 30

In certain circumstances it can be desirable to modulate
(i.e., increase or decrease) the quantity of oxygen in a
mammal, either systemically or locally (e.g., in an organ or
portion thereof, tissue, cells). For example, appropriate
modulation of oxygen levels can be therapeutically benefi- 35
cial for individuals with anemia or oxidative stress, or in
individuals to be treated by local oxygen starvation at the
site of a tumor. Thus, a need exists for methods to modulate
the concentration of oxygen in a mammal.

When macrophages are activated by bacteria, bacterial 40
products, T lymphocyte-derived cytokines, and antigens,
they respond by converting arginine into NO via nitric oxide
synthase. Blocking the synthetic pathway of NO production
has been demonstrated to alleviate immunologically medi-
ated joint destruction that occurs in animal models of 45
arthritis, as well as in glomerulonephritis (McCartney-Fran-
cis, N. et al., *J. Exp. Med.* 178:749-754 (1993); Weinberg,
J. B. et al., *J. Exp. Med.* 179:651-660 (1994)). NO is also
thought to play a role in other inflammatory conditions such
as colitis, iritis and hemodynamic shock. In addition, tumor 50
cells secrete NO to regulate blood flow. Thus, methods of
therapy to reduce the concentration of NO are desirable to
alleviate these conditions.

SUMMARY OF THE INVENTION

The invention relates to the NO consuming and O₂
consuming activity of naturally occurring and variant hemo-
proteins also referred to herein as deoxygenases or hemo-
proteins having deoxygenase activity (also, hemoproteins 60
having NO-consuming activity). Hemoproteins can be iden-
tified as having these enzymatic activities by the methods
described herein used to characterize enzymatic activities. In
particular, the invention relates to the NO-consuming and
deoxygenase activity of *Ascaris* hemoglobin (AH), myoglo- 65
bin and flavohemoglobins (e.g., flavohemoglobins from
bacteria, plants, fungi). One or more hemoproteins can be

used in a method to reduce the concentration of oxygen in
an aqueous solution, by adding to the aqueous solution a
hemoprotein having deoxygenase activity, as well as a
reducing agent, and incubating the resulting solution under
conditions suitable for deoxygenase activity. For NO-en-
hanced deoxygenase activity, depending on the enzyme, NO
or an NO donor can be added to the aqueous solution to
enhance deoxygenase activity under appropriate conditions.
Hemoproteins can also be used to reduce the concentration
of NO in an aqueous solution.

In one aspect, the invention relates to a method of
enzymatically reducing the concentration of oxygen and/or
NO in a mammal. The method comprises administering a
therapeutically effective amount of a hemoprotein with
deoxygenase activity to a mammal in need thereof.

In another aspect, the invention relates to a method of
treating a mammal having a disorder characterized by the
presence of pathologically proliferating cells, such as pros-
tatic hypertrophy, restenosis (as of a coronary artery), psor-
iasis or a tumor. The method comprises administering a
therapeutically effective amount of a hemoprotein with
NO-activated deoxygenase activity to a mammal having
such a condition. In additional embodiments, the invention
relates to methods of treating a mammal having a tumor. In
one embodiment, the invention relates to a method of
deoxygenating a tumor. The method comprises administer-
ing a therapeutically effective amount of a hemoprotein with
deoxygenase activity to a mammal having a tumor. In
another embodiment, the invention relates to a method of
anti-tumor therapy. The method comprises administering a
therapeutically effective amount of a hemoprotein with
deoxygenase activity and a therapeutically effective amount
of a cytotoxic agent to a mammal having a tumor. In a
particular embodiment, the cytotoxic agent is a bioreductive
cytotoxic agent and the deoxygenase can be one that is
activated by NO. In another embodiment, the invention is a
method of potentiating the cytotoxic activity of a bioreduc- 35
tive cytotoxic agent. The method comprises administering a
therapeutically effective amount of a hemoprotein with
NO-activated deoxygenase activity and a therapeutically
effective amount of a bioreductive cytotoxic agent to a
mammal having a tumor. 40

In another aspect, the invention relates to a method of
enzymatically generating toxic reactive oxygen species
(e.g., hydrogen peroxide, superoxide, hydroxyl) for therape-
utic purposes (e.g., a toxic effect in a tumor). The method
comprises administering an effective amount of a hemopro-
tein (e.g., a globin with deoxygenase activity) to a mammal
in need of such therapy.

In another aspect, the invention relates to a composition
comprising a hemoprotein having NO-activated deoxyge-
nase activity and a physiologically acceptable carrier. In one
embodiment, the composition further comprises a cytotoxic
agent (e.g., an anti-tumor agent) and/or a reducing agent. In
a preferred embodiment, the cytotoxic agent is a bioreduc- 55
tive cytotoxic agent.

In another aspect, the invention relates to a method of
reducing the concentration of oxygen in an aqueous solu-
tion, comprising adding a hemoprotein having NO-activated
deoxygenase activity to the solution. Preferably, the solution
contains NO, or NO is added to the solution either directly
or indirectly.

In another aspect, the invention relates to a method of
designing an oxygen lowering enzyme.

The invention further relates to naturally occurring and
mutant hemoproteins with NO-activated deoxygenase activ-
ity, as described herein, for use in therapy (including pro-