PHAGOCYTE LUMINESCENCE: FROM QUANTUM MECHANICAL CONSIDERATIONS TO CLINICAL APPLICATIONS

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The spin quantum number is considered with regard to its influence over oxygen reaction chemistries ranging from chemical combustion to phagocyte oxygenation activity. The importance of spin conservation and its influence on reaction rate is developed with reference to absolute reaction rate theory. Phagocyte luminescence, *i.e.*, photon emission, is the energy product of the highly exergonic oxygenation reactions required for microbicidal action. Photon emission provides information about the intensity of oxygenation activity. If the type and quantity of chemiluminigenic substrate and other reaction conditions are adequately controlled, phagocyte metabolic function can be quantified by measuring the product luminescence. A new luminescence approach for measuring phagocyte opsonin receptor expression is described. The system is designed to analyze the *in vivo* state of immune activation based on the degree of opsonin receptor expression per circulating phagocyte. Complete luminescence measurement of basal and stimulated phagocyte oxidase and oxidase-haloperoxidase activities in combination with analysis of phagocyte opsonin receptor expression requires a drop of blood and is complete in thirty minutes.

Keywords: spin quantum number; combustion; oxygenation; spin conservation; absolute reaction rate theory, phagocyte; luminescence; oxidase; haloperoxidase.

PARSIMONY AND SYMMETRY

Phagocyte luminescence in the visible range of the electromagnetic spectrum was first described over two decades ago[1-3]. Discovery of this phenomenon resulted from testing the hypothesis that phagocytes change the spin quantum number of O_2 and in so doing realize the great reactive potential of O_2 for effecting microbicidal action. The following will present the conceptual foundation and hopefully some noumenal insight into the nature of oxygenation reactions in general and phagocyte microbicidal action in particular.

Two guiding principles have directed my approach to understanding oxidation reactions. The first is the rule of parsimony which is sometime referred to as Occam's razor. In essence, the simplest of competing theories is preferred to the more complex; *i.e.*, the best explanation of a phenomenon requires the least assumptions. Simplicity is truth.

The other principle has to do with the concept of symmetry. Symmetry relates to the property of sameness. Symmetry has to do with those properties that remain invariant under change, *e.g.*, chemical reaction. For any reaction, the symmetry of reactants are expressed in the product symmetries; *i.e.*, the overall symmetry of the reaction must be retained. Symmetry is at the basis of all conservation laws and scientific equations. Just as simplicity is conceptually linked to truth, symmetry is conceptually linked to aesthetics. In fact, symmetry is commonly defined as beauty of form arising from balanced proportions. Symmetry is beauty.

ELECTRON SPIN QUANTUM NUMBER AND MULTIPLICITY

The quantum mechanical concept of electron spin angular momentum grew from initial spectroscopic observations and later theoretical considerations[4]. The spin quantum number, s, is restricted to a value of $+ {}^1/_2$ or $- {}^1/_2$. The total spin quantum number of an atom or molecule, S, is the sum of the individual electron spin quantum numbers. Multiplicity is a spectroscopic term which is based on the total spin state of an atom or molecule; it is defined by the equation $|2S| + 1$. The orbitals of most organic molecules are either completely filled or completely empty, *i.e.*, $S = 0$, and as such, their multiplicity is singlet, *i.e.*, $|2(0)| + 1 = 1$. In keeping with Pauli's exclusion principle, O_2 has one electron in each of its two pi antibonding orbitals (π^*), and in accord with Hund's maximum multiplicity rule, each of these electrons have the same spin value, *i.e.*, $S = {}^1/_2 + {}^1/_2 = 1$ or $- {}^1/_2 + -{}^1/_2 = -1$. Therefore, the multiplicity of O_2 is triplet, *i.e.*, $|2(1 \text{ or } -1)| + 1 = 3$. The multiplicity of the atom or molecule is often represented by the superscript notation, *e.g.*, $^3O_2$.

SYMMETRY CONSERVATION AND ABSOLUTE REACTION RATE

Wigner's spin conservation rule defines the tendency of a system to resist any change in spin angular momentum; *i.e.*, the total spin momentum of a reacting system is conserved[5]. Highly exergonic reactions of O_2 with organic materials do not occur spontaneously, but instead require relatively large activation energies. Absolute reaction rate theory provides an explanation for the low direct reactivity of O_2. According to absolute reaction rate theory,

$$\text{Rate} = \kappa \, (kT/h) \, K^* e^{-Ea/RT} \qquad (1)$$

where κ is the transmission coefficient, (kT/h) is the frequency factor (*i.e.*, k is the Boltzmann constant, T is the absolute temperature and h is Planck's constant), K* is the activation equilibrium constant (*i.e.*, K* = F†/FaFb: where Fa and Fb are the initial states of the reactants and F† is the activated complex), Ea is the activation energy per mole at absolute zero (*i.e.*, approximately the energy of activation in the classical Arrhenius equation), and R is the gas constant[6,7].

Even when a reaction system has the required K* and Ea, crossing the reaction barrier is not guaranteed. The crossing tendency, *i.e.*, the number of activated reaction complexes

yielding products relative to the total number having reached the activated state, is defined by the transmission coefficient, κ. The value of κ is a gauge of reaction allowedness[5,8]. When total symmetry, including spin symmetry, is conserved in the transition from reactants through the reaction complex on to reaction products, the value of κ will approximate unity. However, reactions involving change in spin state typically have κ values of less than 10^{-4}.

The reaction of molecular oxygen (S = 1) with a substrate molecule (S = 0):

$$^3O_2 + {}^1\text{Molecule} \Rightarrow {}^3O_2\text{-Molecule} \quad (2)$$

will produce a S = 1 product as defined by spin conservation. The low κ value associated with such a reaction explains why combustion reactions require high activation energy.

For example, if the S = 0 substrate molecule is H_2, sufficient activation energy must be provided to homolytically cleave its sigma (σ) bond[9],

$$^1H_2 + \text{Energy } (+104\ kcal/mol) \Rightarrow 2\ {}^2H \quad (3)$$

The resulting two S = $^1/_2$ hydrogen atoms produced by this homolytic cleavage, can now react with oxygen in the S 1-$^1/_2$ reaction,

$$^2H + {}^3O_2 \Rightarrow {}^2HO_2 \Leftrightarrow {}^2O_2^{\cdot-} + H^+ \quad (4)$$

to produce S = $^1/_2$ hydrodioxylic acid (perhydroxylic acid) and its dissociation products, a proton and the superoxide anion. Superoxide can react with the other hydrogen atom in a spin annihilation, *i.e.*, S $^1/_2$ - $^1/_2$, reaction:

$$^2H + {}^2HO_2 \Rightarrow {}^1H_2O_2 \quad (5)$$

to yield hydrogen peroxide (S = 0).

Further reaction of the product peroxide requires additional energy for homolytic cleavage,

$$^1H_2O_2 + \text{Energy } (+35\ kcal/mol) \Rightarrow 2\ {}^2HO \quad (6)$$

The resulting products, two S = $^1/_2$ hydroxyl radicals, can react with additional S = $^1/_2$ hydrogen atoms in a S $^1/_2$ - $^1/_2$ annihilation,

$$^2HO + {}^2H \Rightarrow {}^1H_2O \quad (7)$$

to yield water (S = 0) the ultimate product of this combustion.

Based on the heat of reaction calculations[9]:

$$\{2\ H_2\ (+208\ kcal/mol) \Rightarrow 4\ H\} \quad (8)$$
$$\{O_2\ (+119\ kcal/mol) \Rightarrow 2\ O\}$$
$$\{4\ H + 2\ O \Rightarrow 2\ H_2O + (-442\ kcal/mol)\}$$
$$\text{Net free energy } (\Delta G) = -115\ kcal/mol$$

the net reaction is highly exergonic. A free energy of -115 *kcal/mol* is equivalent to an einstein (Avogadro's number) of 250 *nm* photons.

The spin state difference between $^3O_2$ (S = 1) and organic substrate molecules (S = 0) presents a similar barrier to direct reactivity. Despite large exergonicities, these reactions also require relatively large activation energies. For example, the heat of reaction calculations for the oxygen with ethylene reaction are:

$$\{C_2H_4\ (+146\ kcal/mol) \Rightarrow 2\ CH_2\} \quad (9)$$
$$\{O_2\ (+119\ kcal/mol) \Rightarrow 2\ O\}$$
$$\{2\ CH_2 + 2\ O \Rightarrow 2\ CH_2O + (-358\ kcal/mol)$$
$$\text{Net } \Delta G = -93\ kcal/mol$$

This free energy is equivalent to an einstein of 308 *nm* photons. For reaction to occur, energy is required to homolytically cleave the substrate molecule. Homolytic cleavage yields the S = $^1/_2$ organic radical intermediates that can react with S = 1 $^3O_2$ via a S 1- $^1/_2$ radical reaction pathway. When $^3O_2$ is not limiting, the high net exergonicities of these radical-based reactions insure propagation, *i.e.*, the continued homolytic cleavage of substrate molecules required to sustain combustion.

PHAGOCYTE METABOLISM: CHANGING THE SPIN QUANTUM NUMBER OF OXYGEN

The ability of phagocytes to kill microbes is broad and lethal. This microbicidal action requires the metabolic generation of reducing equivalents via the dehydrogenases of the hexose monophosphate shunt and non-mitochondrial consumption of O_2[10-12]. The requirement for O_2 and the microbicidal potential of a controlled combustion-like oxygenation, lead me to hypothesize that phagocytes kill microbes by changing the spin quantum number of O_2 from S = 1 to S = $^1/_2$ and finally to S = 0. In this process $^3O_2$ is first reduced to ^2O_2H which can disproportionate to yield $^1H_2O_2$ and $^1O_2$[1,13]. Changing S to 0 removes the spin barrier to O_2 reactivity and effectively realizes its electrophilic potential for reactivity.

Whereas chemical combustion is based on radicalization of substrate, phagocyte oxygenation is based on deradicalizing O_2. Either pathway satisfies the spin conservation requirements for reaction. The originally proposed phagocyte mechanism for deradicalizing O_2 is illustrated in Figure 1[14]. This schema illustrates the phagocyte process of carbohydrate consumption, redox metabolism and O_2 consumption resulting in oxygenations yielding electronically excited products and photon emission. This phagocyte process mirrors the inverse process of plant photosynthesis in which photons electronically excite chlorophyll resulting in the generation of reduction potential, as required for CO_2-fixation, *i.e.*, carbohydrate synthesis, and oxidation of water to $^3O_2$.

The first step in the process, *i.e.*, the conversion of O_2 from S = 1 to S = $^1/_2$, was thought to be catalyzed by a flavoprotein oxidase, NADPH:O_2 oxidoreductase[2-3]. Activation of this enzyme was known to produce the phagocyte respiratory burst[12]. The reaction catalyzed by the oxidase is as previously described in Equation 4. The reduction of O_2 generates hydrodioxylic acid. This acid has a p*Ka* of 4.8, and as such, oxidase activity also exerts dynamic control over the acidification of the phagocytic vacuole[15].

As the pH of the reaction condition approaches the p*Ka* 4.8, anionic repulsion no longer restricts disproportionation, and hydrodioxylic acid can react with its conjugate base superoxide in a direct S $^1/_2$ - $^1/_2$ annihilation,

$$^2HO_2 + {}^2O_2^{\cdot-} + H^+ \Rightarrow {}^1H_2O_2 + {}^1O_2 \quad (10)$$

Direct reaction will proceed via a S 0 surface, and as such, the reaction products, including O_2, will have a S = 0 state, *i.e.*, singlet multiplicity[16].

This reaction will yield $^3O_2$ if an intermediate S > 0 catalyst, *e.g.*, a metal-based catalyst such as superoxide dismutase, is involved:

$$^2O_2^{\cdot-} + \text{Metal}^n \Rightarrow {}^3O_2 + \text{Metal}^{n-1} \quad (11a)$$

$$^2O_2^{\cdot-} + \text{Metal}^{n-1} \Rightarrow {}^1H_2O_2 + \text{Metal}^n \quad (11b)$$

The stepwise reduction and oxidation of the S > 0 metal catalyst satisfies the spin conservation requirements for mixed production of ground state $^3O_2$ (S = 1) and $^1H_2O_2$ (S =0)[17].

NITRIC OXIDE: ANOTHER S = $^1/_2$ MOLECULE

Contrary to the conclusions of some early publications[18-19], there is a general lack of theoretical and empirical support for

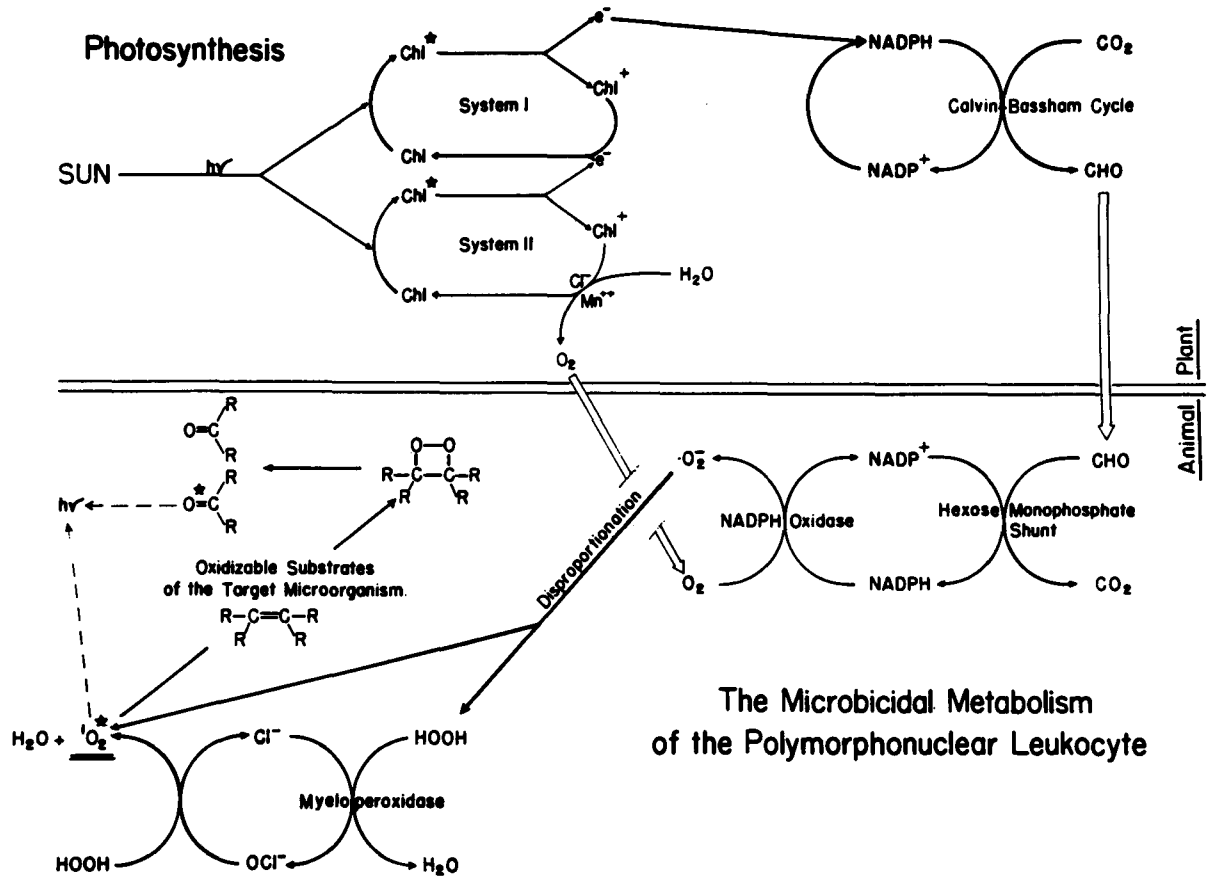

Figure 1

the direct reactivity of $^2O_2^-$ with $S = 0$ biological molecules[20-21]. The commonly held notion that radicals are highly reactive is valid if reaction is with another radical or $S > 0$ reactant, or if the radical has sufficient reactive character, independent of its radical structure, to overcome the orbital overlap restrictions on radical-nonradical reaction. The first condition is met in S $^1/_2$ - $^1/_2$ annihilation reactions, such as the doublet-doublet annihilation of Equation 10. The second condition is met when considering the potent electron extracting capacity of hydroxyl radical (^{2}OH).

The biologic importance of another $S = ^1/_2$ molecule, nitric oxide (^{2}NO), is presently under active investigation. Like $^2O_2^-$, ^{2}NO is being touted as "a free radical that avidly reacts with other molecules"[22]. Unfortunately such generalizations tend to misinform. NO is certainly reactive with other radicals; *i.e.*, ^{2}NO readily participates in S $^1/_2$ - $^1/_2$ annihilation reaction. However, ^{2}NO ($S = ^1/_2$) does not readily react with $S = 0$ molecules. It has been known for over fifty years that a small amount of ^{2}NO can react with and destroy free radicals in a reaction system. ^{2}NO was used to investigate the contribution of free radicals to reaction rate; *i.e.*, a reaction rate determined under low ^{2}NO partial pressure was considered essentially radical reactant-independent[23].

The S $^1/_2$ - $^1/_2$ annihilation reaction between ^{2}NO with $^2O_2^-$ yielding the $S = 0$ peroxynitrite anion,

$$^2NO + {}^2O_2^- \Rightarrow {}^1OONO^- \quad (12)$$

has a reported rate constant of $4 * 10^7$ $M^{-1}s^{-1}$ [24,25]. For comparison, the reaction of $^2HO_2$ with $^2O_2^-$ described in Equation 10 has a reported rate constant of $8 * 10^7$ $M^{-1}s^{-1}$ [26]. The $S = 0$ peroxynitrite product of this reaction may prove to be an important biochemical reactant. Its structure suggests the possibility for electrophilic reactive character similar to $^1O_2$. Reaction of ^{1}OONO$^-$ with a $S = 0$ substrate molecule is spin allowed. It is possible that ^{1}OONO$^-$ could directly dioxygenate an appropriate substrate,

$$^1OONO^- + {}^1Molecule \Rightarrow {}^1O_2\text{-}Molecule + {}^1NO^- \quad (13)$$

In this proposed reaction the peroxy function would serve as an $S = 0$ electrophile and the nitrite function would serve as the leaving group, i.e., nitroxyl anion (^{1}NO$^-$).

Reaction of ^{2}NO with diradical $^3O_2$ is also spin allowed,

$$^2NO + {}^3O_2 \Rightarrow {}^2OONO \quad (14)$$

The product of a S 1 - $^1/_2$ reaction, *i.e.*, a radical propagation reaction, is $S = ^1/_2$. The $S = ^1/_2$ character of ^{2}OONO insures its reactivity with other radicals. Participation in S $^1/_2$ - $^1/_2$ annihilation reactions, *e.g.*, the reaction with an additional ^{2}NO,

$$^2NO + {}^2OONO \Rightarrow {}^1ONOONO \quad (15)$$

is via the S 0 surface and terminates radical propagation by yielding an $S = 0$ product, *e.g.*, $^1N_2O_4$. Homolytic cleavage of $^1N_2O_4$,

$$^1ONOONO + Energy\ (?\ probably < +30\ kcal/mol) \Rightarrow 2\ {}^2NO_2 \quad (16)$$

yields two $S = ^1/_2$ nitrogen dioxides, $^2NO_2$, which can participate in additional radical reactions. Reaction of $S = ^1/_2$ $^2NO_2$ with an additional $S = ^1/_2$ ^{2}NO,

$$^2NO + {}^2ONO \Rightarrow {}^1ONONO \quad (17)$$

yields $S = 0$ $^1N_2O_3$ as product. Both $^1N_2O_4$ and $^1N_2O_3$ are known to participate in nitrosation of $S = 0$ substrates[27,28].

MYELOPEROXIDASE: GENERATION OF HYPOCHLOROUS ACID AND $^1O_2$

Reduction of $^3O_2$ to $^1H_2O_2$ results in some loss of thermodynamic potential but the $S = 0$ product can serve as substrate for myeloperoxidase (MPO) of polymorphonuclear neutrophil leukocytes and monocytes and eosinophil peroxidase (EPO) of eosinophils[10].

These H_2O_2:halide oxidoreductases (XPO's) exert a potent and broad-based microbe killing action which is both halide (X^-) and H_2O_2 dependent[29]. The X^-, H_2O_2 and pH requirements for XPO microbicidal action are probably best appreciated in the context of the Nernstian relationship[30,31]:

$$E = E_o + RT/nF\ ln([Oxidized]/[Reduced]) + RT/nF\ ln[H^+] \quad (18)$$

where **E** is the observed potential in volts, E_o is the standard potential in volts, R is the gas constant, T is the absolute temperature, *n* is the number of electrons per gram-equivalent transferred, ***F*** is a faraday, *ln* [Oxidized]/[Reduced] is the natural log of the ratio of the concentration of reduced to oxidized reactant and *ln* $[H^+]$ is the natural log of the proton concentration.

The XPO reaction can be considered as the combination of two separate redox half reactions: [1] the oxidation of X^- to HOX and [2] the reduction of H_2O_2 to H_2O. The combined reactions can be described in the Nernstian format:

$$\Delta E = EH_2O_2 - EX^- = RT/nF\ ln([H_2O][HOX]/[X^-][H_2O_2][H^+]) \quad (19)$$

As demonstrated in the calculations of Table 1[32], when the X^- is chloride (Cl^-) or bromide (Br^-), the reaction is exergonic over the pH range from 5 to 7. The change in free energy, ΔG, is calculated from the change in potential, ΔE; *i.e.*, $\Delta G = -nF\Delta E$. In the primary reaction, a halide oxidation, exergonicity is inversely related to halogen electronegativity and directly related to $[H^+]$. Also shown in the secondary reaction of Table 1, the product HOX can oxidize an additional molecule of H_2O_2 to yield $^1O_2$. Since the halogen is now the oxidant and H_2O_2 is now the reductant, exergonicity is directly related to halogen electronegativity and inversely related to $[H^+]$.

When H_2O_2 is limiting, the accumulation of HOX and therefore its direct reactivity with the microbe is favored. However, when H_2O_2 is abundant, the generation of $^1O_2$ is favored. Both HOCl and $^1O_2$, the primary and secondary products of XPO activity, are $S = 0$ reactants and can serve as potent microbicidal agents.

Table 1. Effect of pH and halide electronegativity on the free energy of the haloperoxidase reaction:

			Primary Reaction			
			$^1H_2O_2 + {}^1X^- + H^+ \text{ — XPO} \rightarrow {}^1HOX + {}^1H_2O$			
pH	EH_2O_2	-	ECl^-	=	ΔE_1	ΔG_1
5	1.4805	-	1.3465	=	0.1340	-6.16
6	1.4214	-	1.3170	=	0.1044	-4.80
7	1.3623	-	1.2875	=	0.0748	-3.44
pH	EH_2O_2	-	EBr^-	=	ΔE_1	ΔG_1
5	1.4805	-	1.1835	=	0.2970	-13.66
6	1.4214	-	1.1540	=	0.2674	-12.30
7	1.3623	-	1.1245	=	0.2378	-10.94
			Secondary Reaction			
			$^1HOX + {}^1H_2O_2 \longrightarrow H^+ + {}^1X^- + {}^1O_2 + {}^1H_2O$			
pH	EHOCl	-	EH_2O_2	=	ΔE_2	ΔG_2
5	1.3465	-	0.3865	=	0.9600	-21.63
6	1.3170	-	0.3274	=	0.9896	-22.99
7	1.2875	-	0.2683	=	1.0192	-24.35
pH	EHOBr	-	EH_2O_2	=	ΔE_2	ΔG_2
5	1.1835	-	0.3865	=	0.7970	-14.13
6	1.1540	-	0.3274	=	0.8266	-15.49
7	1.1245	-	0.2683	=	0.8562	-16.86
			Net Reaction			
			$2\ {}^1H_2O_2 \longrightarrow {}^1O_2 + 2\ {}^1H_2O$			
pH	EH_2O_2	-	EH_2O_2	=	ΔE_{net}	ΔG_{net}
5	1.4805	-	0.3865	=	1.094	-27.79
6	1.4214	-	0.3274	=	1.094	-27.79
7	1.3623	-	0.2683	=	1.094	-27.79

PHOTON EMISSION FROM OXYGENATION REACTIONS

Light is an energy product of oxygenation reactions, and luminescence is a product of phagocyte microbicidal metabolism. In fact, phagocyte luminescence was discovered in experiments to test the hypothesis that phagocytes change the spin quantum number of O_2 allowing it to serve as a direct dioxygenating agent[1].

For any chemical reaction, certain fundamental requirements must be met. The reaction must be thermodynamically allowed and mechanistically possible, *i.e.*, symmetry allowed. Light emission in the visible range of the spectrum, 700 to 340 *nm*, requires highly exergonic reactions yielding ΔG's of -41 to -84 *kcal/mol*. Reactions of such exergonicity are not common in biochemistry. However, dioxygenation reactions, such as the dioxygenation described in Equation 9, do satisfy these high energy requirements. Changing the spin quantum number of O_2 from $S = 1$ to $S = 1/2$ and finally to $S = 0$ by reduction of $^3O_2$ to ^2O_2H with disproportionation to yield $^1H_2O_2$ and $^1O_2$, provides the mechanistic pathway to reactive dioxygenation.

Phagocyte luminescence reflects the quantity of oxygenating agents generated, the susceptibility of available substrates to oxygenation, and the quantum yield of the substrate oxygenated[33,13]. Native substrates vary in susceptibility and are typically of relatively low quantum yield. Addition of a high quantum yield substrate, *i.e.*, a chemiluminigenic substrate, greatly increases the light yield and defines the product luminescence according to the relationship:

$$d\mathbf{L}/dt = k[\mathbf{X}][\mathbf{C}] \quad (20)$$

where $d\mathbf{L}/dt$ is the luminescence intensity, k is the proportionality constant, [X] is the concentration of oxygenating agent, and [C] is the concentration of chemiluminigenic substrate. When [C] is much greater than [X], *i.e.*, [C]>>>[X], the contribution of C to $d\mathbf{L}/dt$ approaches zero order, *i.e.*, C is not rate limiting, and the equation simplifies to:

$$d\mathbf{L}/dt = k[\mathbf{X}] \quad (21)$$

The chemical nature of C determines the type of oxygenation activity measured. Oxidase activity is measurable as the luminescence resulting from the reductive dioxygenation of the acridinium salt dimethyl biacridinium dinitrate (DBA; lucigenin)[34]. When DBA is C, the luminescence is proportional to the available H_2O_2 under alkaline conditions,

$$^1DBA^{++} + {}^1H_2O_2 \Rightarrow 2\ ^1\text{N-methylacridone} + \text{Photon} \quad (22)$$

Under neutral to mildly acid conditions, DBA can be univalently reduced to the $S = 1/2$ state,

$$^1DBA^{++} + e^- (^2O_2^-) \Rightarrow {}^2DBA^+ + (^3O_2) \quad (23)$$

and can then react with superoxide in a $S\ 1/2 - 1/2$ annihilation via a $S = 0$ surface,

$$^2DBA^+ + {}^2O_2^- \Rightarrow 2\ ^1\text{N-methylacridone} + \text{Photon} \quad (24)$$

yielding a dioxygenated product and ultimately photon emission. DBA does not serve as substrate for measuring HOCl or $^1O_2$, *i.e.*, the products of XPO activity.

Cyclic hydrazides, *e.g.*, luminol, can take several different reactive pathways ultimately resulting in dioxygenation yielding luminescence. In granulocytic leukocytes and monocytes, luminol-dependent luminescence reflects in large part XPO activity via direct reaction of luminol with $^1O_2$,

$$^1\text{Luminol} + {}^1O_2 \Rightarrow {}^1\text{Aminophthalate} + {}^1N_2 + \text{Photon} \quad (25)$$

or via the stepwise S 0 oxidation-reduction reaction[35],

$$^1\text{Luminol} + {}^1HOCl \Rightarrow {}^1\text{Luminol}_{\text{dehydrogenated}} + {}^1Cl^- + {}^1H_2O \quad (26a)$$

$$^1\text{Luminol}_{\text{dehydro.}} + {}^1H_2O_2 \Rightarrow {}^1\text{Aminophthalate} + {}^1N_2 + \text{Photon} \quad (26b)$$

In macrophages and monocytes, $^1OONO^-$, the $S = 0$ product of ^2NO reaction with $^2O_2^-$ as described in Equation 12, might also react with luminol via a S 0 surface,

$$^1\text{Luminol} + {}^1OONO^- \Rightarrow {}^1\text{Aminophthalate} + NO^- + {}^1N_2 + \text{Photon} \quad (27)$$

but the validity of this reaction is not yet established.

THE PHOTON AS INFORMATION: EVALUATING IMMUNE STATUS AT THE SPEED OF LIGHT

The inflammatory response to infection and injury requires the synthesis, transfer, and response to immunologic information in the molecular form of cytokines and other inflammatory agents. Granulocytic leukocytes and monocytes are the microbicidal effector cells of inflammation. In response to inflammatory mediators, these phagocytes undergo progressive activation ultimately directed to phagocytosis and killing of the target microbe.

Inflammatory mediators direct the changes in phagocyte and endothelial cell receptor expression required for phagocyte-endothelial contact, diapedesis, and phagocyte transit through the interstitial space to the site of infection or injury. The increased expression of phagocyte opsonin receptors, *e.g.*, the complement (CD11b/CD18 and CD35) and immunoglobulin receptors, assures effective phagocyte-microbe contact recognition, phagocytosis, degranulation, and activation of respiratory burst metabolism. The resulting redox metabolism generates the oxygenating agents required for microbe killing.

Phagocyte opsonin receptor expression is proportional to the degree of inflammatory mediator exposure. As such, the state of *in vivo* immune activation, *i.e.*, inflammation, is reflected by the opsonin receptor expression per circulating phagocyte. The opsonin receptor expression per phagocyte can be assayed by luminescence measurement of the initial activation of phagocyte oxygenation activity under conditions of controlled opsonin exposure[36].

The relationship of phagocyte opsonin receptor expression to inflammatory mediator exposure can be concisely expressed as,

$$\mathbf{R/P} = k[\mathbf{P}][\mathbf{I}] \quad (28)$$

where **R** represents the opsonin receptor, **P** represents the phagocyte, **R/P** is the opsonin receptor expression per phagocyte, k is the proportionality constant, [P] is the concentration of phagocytes, and [I] is the concentration of inflammatory mediators capable of inducing opsonin receptor expression. When [I] is sufficient to maximally elicit opsonin receptor expression, [I*max*],

$$\mathbf{R}max/\mathbf{P} = k[\mathbf{P}][\mathbf{I}max] \quad (29)$$

the maximum number of opsonin receptors per phagocyte, *i.e.*, **R***max*/**P**, will be expressed.

Phagocyte contact with opsonins, *e.g.*, complement or immunoglobulin coated microbes or particles, activates respiratory burst metabolism yielding oxygenating agents according to the relationship,

$$d\mathbf{X}/dt = k[\mathbf{R/P}][\mathbf{P}][\mathbf{O}] = k[\mathbf{R}][\mathbf{O}] \quad (30)$$

where $d\mathbf{X}/dt$ is the rate of oxygenating agent generation, [O] is the concentration of opsonin stimulus, and the other components are as previously described. When [O]>>>[R], O does not exert a rate limiting effect, and the equation simplifies to,

$$d\mathbf{X}/dt = k[\mathbf{R}] \quad (31)$$

As previously described in Equations 20 and 21, $d\mathbf{L}/dt$ is dependent on [X], and thus, $d\mathbf{L}/dt$ is proportional to $d\mathbf{X}/dt$. Therefore, it follows that,

$$d\mathbf{L}/dt = k[\mathbf{R}] \quad (32)$$

These relationships are practically applied in a commercially available whole blood luminescence method (the AXIS™ System, ExOxEmis, Inc., San Antonio, TX, USA) for assessing *in vivo* inflammation. This method is based on measuring the circulating relative to maximum opsonin receptor expression per circulating phagocyte[37]. Approximately one drop of blood is added to 5 ml of diluting medium. One portion of the diluted blood, equivalent to 1 µl of whole blood, is tested without modification by adding the blood to a test condition containing non-limiting quantities of **O** and **C**. Thus,

$$d\mathbf{L}/dt = k[\mathbf{R}] = k[\mathbf{P}][\mathbf{I}\textit{in vivo}] \quad (33)$$

An equivalent portion of the same diluted blood is tested in the presence of sufficient inflammatory modulator to insure maximum phagocyte opsonin receptor expression, **I***max*, and the same non-limiting quantities of **O** and **C**,

$$d\mathbf{L}max/dt = k[\mathbf{R}max] = k[\mathbf{P}][\mathbf{I}max] \quad (34)$$

As such, the ratio of **L** to **L***max* reflects the ratio of the circulating opsonin receptor expression (**CORE**) to the maximum opsonin receptor expression (**MORE**) per phagocyte. The ratio can also be presented in its reciprocal form as the percentage opsonin receptor reserve.

$$1\text{-}(\mathbf{L}/\mathbf{L}max)*100 = \text{Percentage Opsonin Receptor Reserve } (\%\ \textit{ORR}) \quad (35)$$

The **CORE/MORE**™ ratio or *% ORR* provide a sensitive nonparametric gauge of the systemic state of inflammation. This measurement can be obtained in combination with opsonin receptor-independent measurement of phagocyte functional activity and capacity. The basal and stimulated phagocyte oxidase activity can be measured using DBA as **C** without added stimulus and with low dose (10 *pmol*/test) phorbol-12-myristate-13-acetate (PMA) as the nonopsonin stimulus. In like manner, basal and stimulated phagocyte oxidase-driven haloperoxidase activity can be measured using luminol as **C** without added stimulus and with high dose (5 *nmol*/test) PMA.

The **CORE/MORE** ratio gauges the *in vivo* state of immune activation, and the measurement of basal and stimulated phagocyte oxidase and oxidase-haloperoxidase activities defines the state of functional activation as well as the maximal metabolic capacities of circulating phagocytes. Such information is clinically useful for diagnosis and management of patients with infectious and inflammatory diseases. Luminescence allows rapid, real time analysis. The luminescence method described requires a very small quantity of blood, is relatively inexpensive, and does not employ radioisotopes. The clinical utility of the system is presently being evaluated.

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