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Equations Describing the Reactant Concentration-profiles in Fig. 3.

The following outlines the simplifying assumptions allowing Scheme 3 to be treated as a two-stage consecutive irreversible reaction, with the resulting equations plotted in Fig. 3. The rate constant k_g was defined as described in the Results section, and a pseudo first-order reaction condition was assumed for the reaction between α-TOH and ROO• (i.e., [α-TOH] » [ROO•]). Thus, Scheme 3 was treated as an A ---> B ---> C system where A = R-N=N-R, B = ROO•, and C = α-TO•. [R-N=N=R], [ROO•], and [α-TO•] at time t are now obtained immediately (38):

$$R-N=N-R(t)=R-N=N-R_0\ \mathrm{Exp}[-k_g\ t] \qquad [A1]$$

$$ROO\bullet(t)=\left(\frac{2\ k_g\ R-N=N-R_0}{k_i^{'}-2\ k_g}\right)\left(\mathrm{Exp}[-2\ k_g\ t]-\mathrm{Exp}[-k_i^{'}\ t]\right) \qquad [A2]$$

$$\alpha-TO\bullet(t)=R-N=N-R_0\left(\frac{1}{2}-\frac{1}{2\ \mathrm{Exp}[k_g\ t]}-\left(\frac{2\ k_g}{k_i^{'}-2\ k_g}\right)\left(\mathrm{Exp}[-2\ k_g\ t]-\mathrm{Exp}[-k_i^{'}\ t]\right)\right) \qquad [A3]$$

where $k_i^{'} = k_i \times$ [α-TOH] and the subscript 0 denote concentration at "t = 0", respectively.

Note that the (integrated) differential equations describing the *total* reactant concentration-profiles of R-N=N-R, ROO•, and α-TO• are mathematically *in*dependent of flux terms that describe the partitioning reactions. This can be shown by writing out, for example, $d[\alpha\text{-TO}^\bullet]_{total}/dt = d[\alpha\text{-TO}^\bullet]_{aq}/dt + d[\alpha\text{-TO}^\bullet]_{LDL}/dt$.

To graph the *local* reactant concentration-profiles of α-TO• (see next section for definition of term 'local' concentration), it was assumed that the partitioning reactions were not rate limiting to the chemical reactions characterizing the production of this species (i.e., they were neglected).

Solubilization Dynamics and Partitioning

In Schemes 3 and 4 the lipoprotein particle was treated as a solvent for a given solute (S) which may partition into the particle from the aqueous phase. This partitioning is governed by the following equilibrium:

$$S_{aq} \underset{k_-}{\overset{k_+}{\rightleftharpoons}} S_{LDL} \qquad [A4]$$

where k_+ and k_- denote the entry and exit rate constant into and out of LDL, respectively. The partition coefficient, K_p, is given by:

$$K_p = \frac{k_+}{k_-} = \frac{[S]_{LDL}}{[S]_{aq}} = \frac{[\bar{S}]_{LDL}\ V^f_{LDL}}{[\bar{S}]_{aq}\ (1 - V^f_{LDL})} \qquad [A5]$$

where $[\bar{S}]$, and [S] denote the 'local' and stoichiometric concentration (with respect to the total volume of the LDL suspension) of the solute, respectively. The superscript f denotes the fraction of the total volume. Since $V^f_{LDL} = [LDL] \times \bar{V}_{LDL}$ (see also Eqn 2), the local concentrations of species residing in *either* the aqueous *or* lipoprotein compartment are given by:

$$[\bar{S}]_{aq} = \frac{[S]_{aq}}{(1 - [LDL]\ \bar{V}_{LDL})} \qquad [A6a]$$

and

$$[\bar{S}]_{LDL} = \frac{[S]_{LDL}}{[LDL]\ \bar{V}_{LDL}} \qquad [A6b]$$

Using mass balance and Eqn A5, we find for the local concentrations of species that *partition* across the water-LDL interface; i.e., which reside in *each* of the two compartments:

$$[\bar{S}]_{aq} = \frac{[S]_{tot}}{(1 - [LDL]\ \bar{V}_{LDL})\ (1 + K_p)} \qquad [A7a]$$

and

$$[\bar{S}]_{LDL} = \frac{[S]_{tot}}{[LDL]\ \bar{V}_{LDL} \left(\frac{1}{K_p} + 1 \right)} \qquad [A7b]$$

where $[S]_{tot}$ is the sum of the *stoichiometric* concentrations of the species within the aqueous and lipid phases (i.e., $[S]_{tot} = [S]_{aq} + [S]_{LDL} = [\bar{S}]_{aq}\ (1 - [LDL]\ \bar{V}_{LDL}) + [\bar{S}]_{LDL}\ [LDL]\ \bar{V}_{LDL}$).

Due to the scarcity of data characterizing the dynamics of solubilization and partitioning of molecules into LDL, estimates of k_+, k_-, and hence K_p for R-N=N-R (AMVN or AAPH), R$^\bullet$, ROO$^\bullet$, and ROOH, were inferred from data pertaining to molecules with a comparable number of carbon atoms measured previously in micellar systems (81). The formalism describing the exchange reactions of molecules in micellar suspensions was applied (in analogy) to LDL as follows.

The relationship between the 'mole fraction' coefficient, K_x, and the 'local concentration scale' coefficient, K_{MW}, for small solute incorporation numbers, is (81):

$$K_{MW} = \frac{[\bar{S}]_{LDL}}{[\bar{S}]_{aq}} = \frac{K_x}{\bar{V}_m\ 55.5} \qquad [A8]$$

where the subscript m denotes micelle, and 55.5 is the molar concentration of (solvent) water. Thus, the relationship between K_p and K_{MW} follows from Eqns A5 and A8:

$$K_p = \frac{K_{MW}\ V^f_{LDL}}{(1 - V^f_{LDL})} \qquad [A9]$$

Literature values for K_x were used because they were found to be more abundant than estimates for K_{MW}, and they were transformed into K_{MW}-values using Eqn A8. The values for K_{MW} were then used to estimate k_+ and k_- using Eqns. A9 and A5.

For AAPH we calculate K_{MW} from K_x-values of a singly positively charged molecule into a like charged micelle, given the abundance of positively charged lysine and arginine residues on LDL. The values of K_x of $C_{12}H_{25}N^+(CH_3)_2COOCH_3$ and $C_{12}H_{25}N^+(CH_3)_2CH_2COOCH_3$ are 1.44×10^3 and 1.22×10^3 for positively charged micelles, respectively (see Table 2 in (81)). Using a value of 1×10^3 and $\overline{V}_m = 0.363$ dm^3/mole (81), we obtain $K_{MW} \approx 50$. The particular dependence of the partition coefficient on the number of carbon atoms for alkyl sulfates and their micelles (54) was used to estimate K_{MW} for AAPH and AMVN using an exponential fit and extrapolation to alkyl sulfates with 6 methylene units ($r^2 = 0.999$; data not shown). This procedure yielded a ~180-fold reduction in the partition coefficient of an alkyl sulfate with 6 methylene units when compared with an alkyl chain of 12 methylene groups; thus $R = Me_2CHC(NH_2^+)NH_2$ was treated as having effectively 3 methylene groups. Combining these data yielded $K_{MW} = 50 / 180 \approx 0.3$. Thus, the *local* concentration of AAPH molecules in the aqueous phase was $\geq 3 \times$ that inside LDL. For [LDL] = 1 μM in apoB this corresponds to ≈0.1 % of the AAPH being present in the lipoprotein. Such an equilibrium distribution is consistent with AAPH partitioning ≥ 90% into *highly* negatively charged SDS and linoleic acid micelles (15), because in these micelles and at physiological and low ionic strength, the concentration of the (divalent) counter-ion (i.e., AAPH) may be orders of magnitude higher than that in the 'bulk' phase (82). K_{MW} of AMVN was estimated in analogy to amount to ~5×10^3.

The *dynamics* of solubilization of the reactants in question was inferred from estimates of k_{diff}, β, and K_{MW}, where k_{diff} and β denote the rate constant for diffusion-controlled encounters, and the 'net efficiency' of solute incorporation, respectively, and their product is equal to k_+ (81). β is the fraction of encounters leading to solute incorporation with respect to the total number of "hits" (per unit time) of the solute with the micelle. The rate constant characterizing diffusion-

controlled encounters (for an LDL particle) was estimated from the von Smoluchowski equation (see (54)):

$$k_{diff} = 4 \pi N_A 10^{-3} R_{LDL} D_{solute} \quad [A10]$$

where N_A, R, and D denote Avogadro's number, radius and diffusion coefficient. The values of the latter two parameters were taken to be 1.05×10^{-6} cm and 1.0×10^{-6} cm^2 s^{-1} for LDL and a "typical" small molecule, respectively. Substitution of these values into Eqn A10 yielded $k_{diff} = 8 \times 10^9$ M^{-1} s^{-1}. The value of β was estimated to be ≈1% (see Table 4 in (81)), resulting in $k_+ = 8 \times 10^7$ M^{-1} s^{-1}; thus, strictly speaking k_+ is a bimolecular rate constant, but it was taken here to be a (pseudo) first-order rate constant, because LDL was treated as a solvent for molecules to "dissolve" in (see Eqn A4). There appears to be no clear trend in the values of the entry rate constants of different molecules (81); therefore, the value 8×10^7 M^{-1} s^{-1} has been used for the species R-N=N-R, R•, ROO•, and ROOH. Values of k_- for the corresponding AAPH- and AMVN-species amounted to 3×10^8 s^{-1} and 2×10^4 s^{-1}, respectively (i.e., 8×10^7 / 0.3 and 8×10^7 / 5×10^3). The forward (k_+) and reverse (k_-) rate constants correspond to flux terms with respect to *local* concentrations ($k \times [\bar{\ }]$); i.e., the format in which they appear in the differential equations describing the time-evolution of the reactants. Multiplication of these flux terms by their corresponding volume fractions (see Eqns 1 and 2) then yielded the "true" rate constants corresponding to stoichiometric concentrations (see Eqn A2).

AAPH and AMVN-derived alkyl (peroxyl) radicals consist of half the number of carbon atoms of the parent molecule. Hence, we estimated (as before) $K_{MW} \approx 0.02$ for 1/2 AAPH•, and $K_{MW} \approx 5 \times 10^2$ for 1/2 AMVN•; these estimates correspond to k_--values of 4×10^9 s^{-1} and 2×10^5 s^{-1}, respectively. For reasons of simplicity, we assumed that the values of the corresponding ROO• and ROOH species were the same.

The rate constant of oxygen exit from LDL was estimated to be ~5×10^5 s^{-1}; i.e., approximately the mean of the values measured in micelles (10^7 - 10^8 M^{-1} s^{-1}; (81) and references therein) and human erythrocytes (~10^4 s^{-1}; (83)). Using $K_{MW} = 3$ (phosphatidylcholine liposomes; (84)) yielded $k_+ \approx 1.5 \times 10^6$ s^{-1}. It was assumed that the dynamics of partitioning of the superoxide anion radical ($O_2^{\bullet -}$) was of a similar order of magnitude, but given the negative charge of the molecule, we took its K_{MW} to be 0.1, and hence the dynamics to be characterized by $k_+ \approx 5 \times 10^5$ s^{-1} and $k_- \approx 5 \times 10^6$ s^{-1}.

Finally, the mean residence lifetime that characterizes the effective partitioning of α-TOH in membranes may be inferred to be of the order of ns (50). For reasons of simplicity, we took the ratio of the time-averaged local concentration of α-TOH and α-TO• in the aqueous and lipid phases to be unity.

References

(81) Sepulveda, L., Lissi, E., and Quina, F. (1986) Interactions of neutral molecules with ionic micelles. *Adv. Colloid Interface Sci.* **25**, 1-57.

(82) McLaughlin, S. (1989) The electrostatic properties of membranes. *Ann. Rev. Biophys. Biophys. Chem.* **18**, 113-136.

(83) Holland, R. A. B., Shibata, H., Scheid, P., and Piiper, J. (1985) Kinetics of O_2 uptake and release by red cells in stopped-flow apparatus: effects of unstirred layer. *Respir. Physiol.* **59**, 71-91.

(84) Subczynski, W. K., and Hyde, J. S. (1983) Concentration of oxygen in lipid bilayers using a spin-label method. *Biophys. J.* **41**, 283-286.