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Ferrous Iron Release from Transferrin by Human Neutrophil-Derived Superoxide Anion: Effect of pH and Iron Saturation

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The ability of superoxide anion (O_2^-) from stimulated human neutrophils (PMNs) to release ferrous iron (Fe^{2+}) from transferrin was assessed. At pH 7.4, unstimulated PMNs released minimal amounts of O_2^- and failed to facilitate the release of Fe^{2+} from holosaturated transferrin. In contrast, incubation of phorbol myristate acetate (PMA)-stimulated PMNs with holosaturated transferrin at pH 7.4 enhanced the release of Fe^{2+} from transferrin eightfold in association with marked generation of O_2^-. The release of Fe^{2+} was inhibited by addition of superoxide dismutase (SOD), indicating that the release of Fe^{2+} was dependent on PMN-derived extracellular O_2^-. In contrast, at physiologic pH (7.4), incubation of transferrin at physiological levels of iron saturation (e.g. 32%) with unstimulated or PMA stimulated PMNs failed to facilitate the release of Fe^{2+}. The effect of decreasing the pH on the release of Fe^{2+} from transferrin by PMN-derived O_2^- was determined. Decreasing the pH greatly facilitated the release of Fe^{2+} from both holosaturated transferrin and from transferrin at physiological levels of iron saturation by PMN-derived O_2^-. Release of Fe^{2+} occurred despite a decrease in the amount of extracellular O_2^- generated by PMNs in an acidic environment. These results suggest that transferrin at physiologic levels of iron saturation may serve as a source of Fe^{2+} for biological reactions in disease states where activated phagocytes are present and there is a decrease in tissue pH. The unbound iron could participate in biological reactions including promoting propagation of lipid peroxidation reactions or hydroxyl radical formation following reaction with phagocytic cell-derived hydrogen peroxide.

In recent years there has been increasing evidence that iron plays an important role in promoting tissue injury at sites of inflammation (1). This may occur secondary to the promotion of lipid peroxidation reactions or iron-catalyzed hydroxyl radical (·OH) formation via a Fenton reaction. However, under normal physiologic conditions, free iron does not exist in plasma or extracellular fluids and is not readily available for participation in biological reactions. Iron (Fe^{3+}) is carried in the vertebrate bloodstream bound to transferrin in a ternary complex involving iron, transferrin, and bicarbonate (2–6) and is unable to promote oxidant-induced tissue injury in this bound state.

A most challenging problem in transferrin chemistry is the identification of mechanisms by which transferrin is induced to release iron (7). Reduction of Fe^{3+} to ferrous iron (Fe^{2+}), which binds weakly to transferrin, is one mechanism for the release of free iron from transferrin (3, 4, 8). The susceptibility of transferrin-bound Fe^{3+} to reduction can be enhanced by multiple factors. Under conditions of increasing hydrogen ion concentration, the Fe^{3+} · transferrin · bicarbonate complex experiences a conformational change, resulting in a decrease in binding affinity of transferrin for iron (2, 9–11). Theoretically, Fe^{3+}, which is buried in the protein at physiologic pH (7.4), becomes more accessible to reduction, following conformational changes resulting from interaction of transferrin with hydrogen ion (12–14).

Phagocytic cells, including human polymorphonuclear leukocytes (PMNs),[2] can release reactive oxygen metabolites including superoxide anion (O_2^-) following activation (15). Although there have been extensive *in vitro* studies examining the ability of reducing agents like sodium di-

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[2] Abbreviations used: PMN, polymorphonuclear leukocytes; HBSS, Hanks' balanced salt solution; PBS, phosphate-buffered saline; SOD, superoxide dismutase; BPS, bathophenanthroline disulfonate; NTA, nitrilotriacetic acid; BSA, bovine serum albumin; TRF, transferrin; APOTRF, apotransferrin.

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thionite (3), thioglycolate (3), and ascorbate (6) to facilitate the release of Fe^{2+} from transferrin, the ability of PMN-derived O_2^- to facilitate the release of Fe^{2+} from Fe^{3+} · transferrin · HCO_3^{2-} has not been thoroughly investigated (16). In the following *in vitro* experiments we assessed the effect of iron saturation and decreasing pH on the ability of PMN-derived O_2^- to facilitate the release of Fe^{2+} from transferrin.

MATERIALS AND METHODS

Chemicals. All chemicals were purchased from Sigma Chemical Co. (St. Louis, MO) unless otherwise noted.

Isolation of PMNs. Human PMNs were isolated from citrated blood as described previously (17, 18). Briefly, PMNs were obtained from whole blood by Ficoll Hypaque (Pharmacia, Piscataway, NJ) density gradient centrifugation, dextran sedimentation, and hypotonic lysis of red cells. Purified PMNs were resuspended at 10^6 cells/ml in Hanks' balanced salt solution (HBSS). In selected experiments, cells were suspended in phosphate-buffered saline (PBS) containing 1 mM calcium, 0.5 mM magnesium, and 0.1% dextrose.

Superoxide anion generation. Extracellular superoxide anion generation by PMNs was measured spectrophotometrically by the superoxide dismutase (SOD) inhibitable reduction of ferricytochrome *c* (19). PMNs (3×10^6) were suspended in 3 ml HBSS containing 160 μM ferricytochrome *c* and stimulated with phorbol myristate acetate [PMA (100 ng/ml final)]. The rate of extracellular O_2^- generated/min was determined by the change in absorbance/min at 550 nm of PMN cell supernatants ± SOD (90 U/ml final). The difference in absorbance per sample ± SOD divided by the extinction coefficient (21.1×10^3 M^{-1} cm^{-1}) yielded nanomoles $O_2^-/10^6$ PMNs/min. All spectrophotometric measurements were conducted with a dual beam Cary 210 spectrophotometer (Varian Instruments, Palo Alto, CA). The lower limit of AU change that can be measured accurately under conditions in these experiments is 0.001.

Release of Fe^{2+} from holosaturated transferrin. The release of Fe^{2+} from holosaturated transferrin by PMN-derived O_2^- was monitored spectrophotometrically (535 nm) as described previously (3). Briefly, unstimulated and stimulated [PMA (100 ng/ml final)] PMNs (10^6) were incubated in 1 ml HBSS containing iron-saturated transferrin (0.060 mM final), bathophenanthroline disulfonate (BPS) (0.50 mM final) with and without SOD (90 U/ml final) for 20 min in a 37°C shaking water bath. Catalase (100 U/ml) was included in the reaction to inhibit the potential oxidation of Fe^{2+} by hydrogen peroxide. The samples were centrifuged (300*g*, 10 min, 20°C) and the cell supernatants removed. The O_2^--dependent release of Fe^{2+} was determined by the difference in absorbance at 535 nm of samples with and without SOD. The difference in absorbance was divided by the extinction coefficient (2.214×10^4 M^{-1} cm^{-1}) yielding nanomoles $Fe^{2+}/10^6$ PMNs/20 min. In selected experiments, holosaturated transferrin was initially dialyzed against 0.1 M sodium perchlorate ($NaClO_4$) as described previously (20) to remove any contaminating iron and dialyzed against PBS prior to use.

Preparation of transferrin at physiological levels of iron saturation. Transferrin at physiologic levels of iron saturation (i.e., 30–44%) was prepared according to previously published methods (21) with minor modifications. Briefly, a 0.2 mM Fe^{3+} stock solution was prepared by reacting Fe^{3+} with nitrilotriacetic acid (NTA) at a ratio of 1:4 (21). Sideroferrin (0.015 mM) in 0.01 M Tris buffer (pH 7.4) was incubated with Fe · NTA (0.010 mM final) in the presence of sodium bicarbonate ($NaHCO_3$; 10 mM final) for 30 min at 22°C. The percentage iron saturation of the transferrin was determined by comparison of the change in absorbance at 470 nm of the solution to that of holosaturated transferrin (0.015 mM). The partially iron-saturated transferrin was dialyzed in PBS (pH 7.4) containing $NaClO_4$ for 24 h to chelate any extraneous iron (20). The transferrin solution containing $NaClO_4$ was dialyzed against PBS for 12 h concentrated in an Amicon concentrator, and dialyzed again in PBS (pH 7.4) containing 1 mM calcium, 0.5 mM magnesium, and 0.1% dextrose for 12 h. The protein content of the partially iron-saturated transferrin was assessed via the Bradford protein assay with bovine serum albumin (BSA) as a standard (22). Transferrin, at physiologic levels of iron saturation, was then utilized in assays using methodology previously described for holosaturated transferrin.

Release of Fe^{3+} from transferrin. The release of Fe^{3+} from transferrin by conditioned media from unstimulated and PMA-stimulated PMNs was determined. PMNs (2.5×10^6/ml) were incubated with SOD (90 U/ml final) and catalase (100 U/ml final) in the presence and absence of PMA (100 ng/ml final) for 10 min. Following centrifugation (300*g*, 10 min, 22°C), PMN-conditioned media (10^6 cell equivalents) was incubated with transferrin (0.060 mM final) at specific pH values in the presence of deferoxamine (10 mM final) in a shaking water bath (37°C, 20 min). The release of ferric iron from transferrin was monitored spectrophotometrically by the decrease in absorbance of transferrin at 295 nm, as previously described (6).

Statistical analysis. All data represent the mean ± the standard error of the mean (SEM) from at least three experiments. PMNs used in a given experiment were from a single healthy donor. Different donors were used in each experiment. A one factor analysis of variance (ANOVA) and Scheffe F test were performed to compare differences between treatment groups. In appropriate experiments, the Student's paired *t* test was used to compare differences between treatment groups. ($P < 0.05$ was considered significant).

RESULTS

The ability of PMNs to secrete O_2^- at pH 7.4 in response to stimulation with PMA (100 ng/ml final) was determined. While unstimulated PMNs secreted negligible amounts of O_2^- (<1 nmol/min), following stimulation with PMA (100 ng/ml final), the initial rate of O_2^- secreted by 10^6 PMNs was 12.04 ± 0.77 nmol O_2^-/min ($n = 8$). This is consistent with previously published reports (23). The effect of the presence of transferrin on PMN respiratory burst was also determined. Results of these experiments (data not shown) indicate that inclusion of transferrin in the reaction does not alter the rate of oxygen consumption by unstimulated or PMA-stimulated PMNs.

The ability of unstimulated and PMA-stimulated PMNs to facilitate the release of Fe^{2+} from holosaturated transferrin at pH 7.4 was assessed (Table I). While unstimulated PMNs facilitated the release of minimal amounts of iron (0.11 nmol) from holosaturated transferrin (98%), stimulation of PMNs with PMA (100 ng/ml, final) enhanced the release of Fe^{2+} from transferrin approximately eightfold. Dialysis of holosaturated transferrin in 0.1 M $NaClO_4$ prior to use did not significantly alter the ability of PMA-stimulated PMNs to facilitate the release of Fe^{2+} from transferrin (data not shown). Similar results were also obtained in reactions in the absence of catalase (data not shown). Addition of SOD (90 U/ml) to PMA-stimulated PMNs prior to incubation resulted in the release of a small amount of iron (0.17 nmol) from holosaturated transferrin, while incubation of PMA-stimulated PMNs with heat-inactivated SOD (80°C, 60 min) failed to significantly inhibit the release of Fe^{2+} from transferrin by PMA-stimulated PMNs ($P > 0.05$). These results suggest that the release of Fe^{2+} from holosaturated

TABLE I

Effect of Stimulated PMNs on Fe^{2+} Release From Transferrin at pH 7.4

Cells	Transferrin (% iron saturation)	Nanomoles Fe^{2+} released	% Total iron released
PMN	98	0.11 ± 0.03	0.09
PMN + PMA	98	0.82 ± 0.09*	0.70
PMN + PMA + SOD	98	0.17 ± 0.00**	0.14
PMN + PMA + Heated SOD	98	0.63 ± 0.09	0.54
PMN	32	0.06 ± 0.06	0.16
PMN + PMA	32	0.07 ± 0.07	0.18

Note. Iron-saturated transferrin [0.060 mM transferrin; 98% saturated (117.6 μM iron total)] and partially iron-saturated transferrin [0.060 mM transferrin; 32% saturated (38.4 μM iron total)] were incubated with 10^6 unstimulated and stimulated [PMA (100 ng/ml)] PMNs and nmol of Fe^{2+} released/20 min were determined. The effect of SOD (90 U/ml) and heat-inactivated SOD [80°C, 60 min (90 U/ml)] on the release of Fe^{2+} from holosaturated transferrin by stimulated PMNs was also assessed.

* Significantly different from unstimulated PMN.

** Significantly different from PMA-stimulated PMNs (ANOVA; $P < 0.05$).

transferrin by PMNs was dependent on PMN-derived O_2^-. Furthermore, the ability of PMNs to competitively bind Fe^{2+}, resulting in a potential decrease in the amount of Fe^{2+} bound to BPS and an underestimation of the amount of Fe^{2+} released from transferrin by PMN-derived O_2^- was also determined. PMNs were incubated with BPS and a known concentration of Fe^{2+}. The amount of Fe^{2+} chelated to BPS in PMN-conditioned media was then compared to that bound to BPS that had been incubated with the same concentration of Fe^{2+} in the absence of PMNs. Results of these experiments (data not shown) indicate that the same concentration of Fe^{2+} is bound to BPS in reactions in the presence and absence of PMNs, indicating that PMNs do not bind significant amounts of Fe^{2+}.

The ability of PMN-derived extracellular O_2^- to facilitate the release of Fe^{2+} from transferrin at physiologic levels of iron saturation (32%) and pH 7.4 was determined. In contrast to holosaturated transferrin, incubation of unstimulated or PMA-stimulated PMNs with partially iron-saturated transferrin failed to increase the release of Fe^{2+} at physiologic pH (Table I).

The effect of decreasing the pH (by addition of HCl) on the release of Fe^{2+} from transferrin was assessed (Table II). No Fe^{2+} is released from transferrin at pH 6.6 or 6.2 in the absence of a reducing agent. While incubation of unstimulated PMNs at either pH 6.6 or 6.2 with holosaturated transferrin (98%) resulted in the release of minimal amounts of Fe^{2+} (<1 nmol), the amount of Fe^{2+} released from holosaturated transferrin at these mildly acidic pHs was markedly enhanced following incubation with PMA-stimulated PMNs. Fe^{2+} release was inhibited following incubation of PMNs with SOD. In contrast, addition of heat-inactivated SOD failed to significantly inhibit Fe^{2+} release from holosaturated transferrin by PMA-stimulated PMNs at pH 6.6 and 6.2 (data not shown). Incubation of PMA-stimulated PMNs at pH 6.6 or 6.2 with transferrin at physiologic levels of iron saturation (39.63% ± 2.02) resulted in the release of 1.15 ± 0.27 nmol and 1.82 ± 0.48 nmol of Fe^{2+}, respectively (Table II). The increased release of Fe^{2+} from partially

TABLE II

Effect of Decreasing the pH and Superoxide Dismutase on Fe^{2+} Release by PMNs

Cells	Transferrin (% iron saturation)	pH	Nanomoles Fe^{2+} released	% Total iron released
PMN	98	6.6	0.77 ± 0.27	0.65
PMN + PMA	98	6.6	6.66 ± 0.59*	5.66
PMN + PMA + SOD	98	6.6	0.41 ± 0.17**	0.35
PMN	98	6.2	0.54 ± 0.27	0.46
PMN + PMA	98	6.2	5.53 ± 0.69*	4.70
PMN + PMA + SOD	98	6.2	0.48 ± 0.26**	0.41
PMN	39	6.6	0.28 ± 0.08	0.60
PMN + PMA	39	6.6	1.15 ± 0.27*	2.46
PMN + PMA + SOD	39	6.6	0.11 ± 0.11**	0.24
PMN	39	6.2	0.38 ± 0.07	0.81
PMN + PMA	39	6.2	1.82 ± 0.48*	3.89
PMN + PMA + SOD	39	6.2	0.07 ± 0.04**	0.15

Note. Iron-saturated transferrin [0.060 mM, 98% saturated (117.6 μM iron total)] and partially saturated transferrin [0.060 mM, 39% saturated (46.80 μM iron total)] were incubated with 10^6 unstimulated and stimulated [PMA (100 ng/ml)] PMNs at pH 6.6 and 6.2 and nmol of Fe^{2+} released/20 min were determined. The effect of SOD (90 U/ml) on the release of Fe^{2+} from transferrin by stimulated PMNs was also assessed.

* Significantly different from unstimulated PMNs.

** Significantly different from PMA-stimulated PMNs (ANOVA; $P < 0.05$).

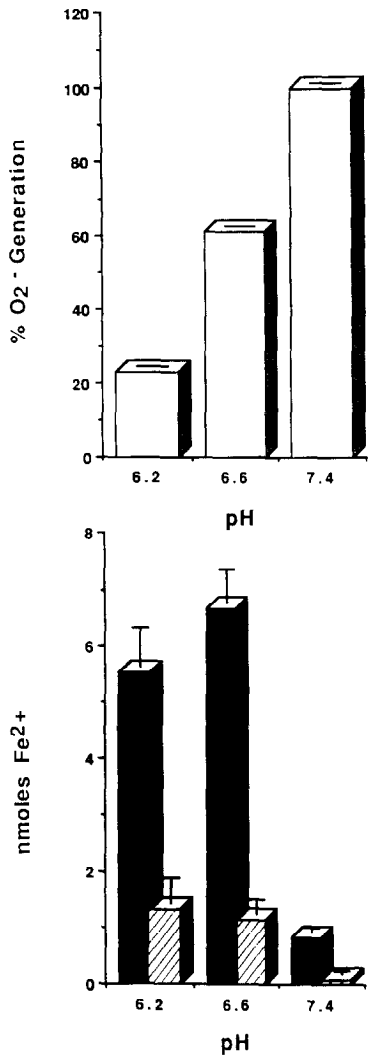

FIG. 1. Effect of decreasing pH on nanomoles of iron released from transferrin and superoxide anion production by PMA-stimulated PMNs. ■, nmole Fe^{2+} released from holosaturated transferrin [0.060 mM; 98% iron saturated (117.6 μM iron total)]; ▨, nmol Fe^{2+} released from partially saturated transferrin [0.060 mM; 32–34% iron saturated (38.4–40.8 μM)], □, $\%O_2^-$ generated by 10^6 PMA-stimulated PMNs (100% = 167 nmol $O_2^-/10^6$ PMA-stimulated PMNs/20 min at pH 7.4).

saturated transferrin at pH 6.6 and 6.2 was also inhibited by SOD (Table II) and occurred despite a pH-dependent decrease in extracellular O_2^- generation (Fig. 1).

In further experiments, a more detailed study on the effect of decreasing pH on the release of ferrous iron from transferrin at physiologic levels of iron saturation was performed. For these studies, PMA-stimulated neutrophils were incubated with transferrin at physiologic levels of iron saturation (39% saturated) at 0.2 pH increments between pH 6.6 and 7.4. At pH 6.8, 0.59 nmol of Fe^{2+} was released by PMA-stimulated neutrophils compared to 0.28 nmol of Fe^{2+} released from transferrin in the presence of unstimulated neutrophils. At pH 7.0, 7.2, and 7.4 PMA-stimulated PMNs did not facilitate the release of enhanced amounts of Fe^{2+} from transferrin. Results of these experiments indicate that neutrophil-derived superoxide ion facilitates the release of enhanced amounts of ferrous iron from transferrin at pHs equal to and less than pH 6.8.

To determine if PMN-derived O_2^- facilitated the release of Fe^{3+} from transferrin, unstimulated and PMA-stimulated PMNs were incubated with holosaturated transferrin and deferoxamine (a Fe^{3+} chelator) in the presence and absence of SOD. The release of Fe^{3+} from holosaturated transferrin was assessed spectrophotometrically by the formation of a Fe^{3+}–deferoxamine complex in PMN-conditioned media. Results of these experiments (data not shown) indicate that equivalent amounts of Fe^{3+} are released from transferrin by unstimulated and PMA-stimulated PMNs. To further address the ability of secretory products derived from PMA-stimulated PMNs to enhance Fe^{3+} release from transferrin, conditioned media from unstimulated and PMA-stimulated PMNs was incubated with iron-saturated transferrin (98%) and partially saturated transferrin (34% ± 1.73) in the presence of deferoxamine (Table III). As indicated by the decrease in absorbance of transferrin at 295 nm, conditioned media from unstimulated or PMA-stimulated PMNs was equally effective in facilitating the release of Fe^{3+} from transferrin, suggesting that secretory products of stimulated PMNs do not effect the release of Fe^{2+} from transferrin at mildly acidic pH by enhancing Fe^{3+} labilization. Furthermore, this data suggests that approximately four times more labile Fe^{3+} is available from holosaturated transferrin than from partially saturated transferrin at pH 7.4. As the pH

TABLE III

Effect of PMN Supernatants on Fe^{3+} Release from Transferrin

PMN-Conditioned media	% Saturation	pH	Decrease in absorbance 295 nm
Unstimulated	98	7.4	0.095 ± 0.048
PMA Stimulated	98	7.4	0.122 ± 0.027 NS
Unstimulated	98	6.6	0.631 ± 0.065
PMA Stimulated	98	6.6	0.549 ± 0.048 NS
Unstimulated	98	6.2	0.836 ± 0.049
PMA Stimulated	98	6.2	0.800 ± 0.044 NS
Unstimulated	34	7.4	0.025 ± 0.025
PMA Stimulated	34	7.4	0.033 ± 0.009 NS
Unstimulated	34	6.6	0.182 ± 0.018
PMA Stimulated	34	6.6	0.158 ± 0.024 NS
Unstimulated	34	6.2	0.182 ± 0.015
PMA Stimulated	34	6.2	0.152 ± 0.043 NS

Note. Iron-saturated transferrin [0.060 mM, 98% saturated (117.6 μM iron total)] and partially saturated transferrin [0.060 mM, 34% saturated (40.8 μM iron total)] were incubated with conditioned media from unstimulated and stimulated [PMA (100 ng/ml)] PMNs (10^6 equivalents) in the presence of deferoxamine (10 mM final) and the decrease in absorbance (295 nm) of transferrin/20 min was assessed. NS = no significant difference between unstimulated and stimulated PMN-conditioned media.

is decreased, significantly more Fe^{3+} is labilized from transferrin due to pH-dependent conformational changes in the transferrin · iron · bicarbonate complex (6).

DISCUSSION

PMN-derived O_2^- facilitated the release of Fe^{2+} from iron-saturated transferrin at physiologic pH, yet failed to facilitate the release of Fe^{2+} from transferrin at physiologic levels of iron saturation at pH 7.4. This is in agreement with a previous report by Biemond *et al.* (16) which suggests that at physiologic pH, stimulated PMNs failed to mobilize iron from partially saturated (60%) transferrin. Furthermore, previous studies at physiologic pH by Aruoma *et al.* (24) provide indirect evidence that is consistent with the hypothesis that O_2^-, in the presence of EDTA, only minimally mobilizes iron from saturated transferrin. Other studies have shown that O_2^- does not directly react with 50% iron-saturated transferrin (25). As a result of these findings, the role of transferrin as a source of iron for biological reactions at sites of acute inflammation has been questioned (16, 26).

As an extension of these studies, we determined the effect of decreasing the pH on the ability of PMN-derived O_2^- to facilitate the release of Fe^{2+} from holosaturated transferrin and from transferrin at physiologic levels of iron saturation. It has been demonstrated that iron release from transferrin can be modulated by several factors including pH (2, 3, 6, 27). As the pH is decreased, the stability of the Fe^{3+} transferrin · bicarbonate complex decreases, rendering the metal susceptible to reduction (3). We found that decreasing the pH to 6.6 and 6.2 facilitated the release of Fe^{2+} from holosaturated transferrin by unstimulated and PMA-stimulated PMNs. The enhanced release of Fe^{2+} under mildly acidic conditions occurred despite a decrease in the amount of O_2^- generated by PMA-stimulated cells, indicating an increased efficiency in O_2^--dependent reduction of Fe^{3+}. Our results are consistent with those of Saito *et al.* (26) who concluded that the amount of Fe^{2+} released from holosaturated transferrin by xanthine–xanthine oxidase-derived O_2^- was greatly enhanced by decreasing the pH.

Of potentially greater biological significance, results of our studies indicate that decreasing the pH facilitated the release of Fe^{2+} from partially saturated transferrin by PMN-derived O_2^-, achieving concentrations in our assay system of greater than 1 μM. Since the plasma concentration of transferrin is approximately 25–35 μM (28), these data suggest that similar concentrations of Fe^{2+} may be released from transferrin at sites of acute inflammation due to PMN-derived O_2^--dependent reduction of labilized Fe^{3+}. Furthermore, comparable amounts of Fe^{2+} were released from partially saturated transferrin by PMA-stimulated PMNs at pH 6.6 and 6.2, despite a progressive decrease in the amount of O_2^- generated by PMA-stimulated PMNs as the acidity increased. This indicates an increased efficiency of Fe^{2+} release from partially saturated transferrin by PMN-derived O_2^- in mildly acidic conditions similar to that observed with holosaturated transferrin. Thus, we suggest that, at sites of tissue injury, the release of Fe^{2+} from transferrin at physiologic levels of iron saturation by PMN-derived O_2^- is dependent on the hydrogen ion-mediated destabilization of the Fe^{3+} · transferrin · bicarbonate complex making the Fe^{3+} more susceptible to reduction.

Inflammation/ischemia

$$Fe^{3+} \cdot TRF \cdot CO_3^{2-} \xrightarrow{H^+} [Fe^{3+} \cdot TRF \cdot CO_3^{2-}] \xrightarrow{\text{PMN-derived } O_2^-} APOTRFCO_3^{2-} + Fe^{2+},$$

where TRF and APOTRF are transferrin and apotransferrin, respectively, and $[Fe^{3+} \cdot TRF \cdot CO_3^{2-}]$ indicates the destabilized conformational state of transferrin.

In conclusion, transferrin at physiologic levels of iron saturation can serve as a source of Fe^{2+} for participation in biological reactions including propagation of lipid peroxidation and generation of hydroxyl radical formation following reaction with phagocytic cell-derived hydrogen peroxide. The restriction of this effect to conditions of mildly acidic pH and the presence of a reducing agent like PMN-derived O_2^- limits its pathophysiologic role to disease processes that result in a significant decrease in tissue pH [e.g., sites of inflammation (29), ischemic injury (30)] or within the microenvironment of activated phagocytic cells where the pH at the cell surface is less than 6.0 (31).

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REFERENCES

1. Aust, S., Morehouse, L., and Thomas, C. (1985) *J. Free Rad. Biol. Med.* **1,** 3 25.
2. Carver, F., and Frieden, E. (1978) *Biochemistry* **17,** 167–172.
3. Kojima, N., and Bates, G. (1979) *J. Biol. Chem.* **254,** 8847–8854.
4. Harris, D., Rinehart, A., Hereld, D., Schwartz, R., Burke, F., and Salvador, A. (1985) *Biochim. Biophys. Acta.* **838,** 295–301.
5. Hubers, H., and Finch, C. (1987) *Physiol. Rev.* **67,** 520–528.
6. Morgan, E. (1979) *Biochim. Biophys. Acta* **580,** 312–326.
7. Braughler, J., Duncan, L., and Chase, R. (1986) *J. Biol. Chem.* **261,** 10,282–10,289.
8. Gaber, B., and Aisen, P. (1970) *Biochim. Biophys. Acta* **221,** 228–233.
9. Aisen, P., Leibman, A., and Zweier, J. (1978) *J. Biol. Chem.* **253,** 1930–1937.
10. Morgan, E., Huebers, H., and Finch, C. (1978) *Blood* **52,** 1219–1228.
11. Lestas, A. (1976) *Brit. J. Haematol.* **32,** 341–350.
12. Ketchmar, S., and Raymond, K. (1986) *J. Amer. Chem. Soc.* **108,** 6212–6218.
13. Konopka, K., Bindereif, A., and Nieland, J. (1982) *Biochemistry* **21,** 6503–6508.

14. Bates, G. (1981) Fifth International Conference on Protein, Iron Storage, and Transport, San Diego and La Jolla, CA.
15. Fantone, J., and Ward, P. (1982) *Amer. J. Pathol.* **107,** 397–418.
16. Biemond, P., van Eijk, H., Swaak, A., and Koster, J. (1984) *J. Clin. Invest.* **73,** 1576–1579.
17. Salin, M., and McCord, J. (1974) *J. Clin. Invest.* **54,** 1005–1009.
18. Boyum, A. (1967) *Scand. J. Clin. Lab. Invest.* **21**(Suppl. 97), 77–89.
19. Babior, B., Kipnes, R., Curnutte, J. (1972) *J. Clin. Invest.* **52,** 741–744.
20. Baldwin, D., Jenny, E., and Aisen, P. (1984) *J. Biol. Chem.* **259,** 13,391–13,394.
21. Graham, G., and Bates, G. (1976) *J. Lab. Clin. Med.* **88,** 477–486.
22. Bradford, M. (1976) *Anal. Biochem.* **72,** 248–254.
23. Guthrie, L. A., McPhail, L. C., Henson, P. M., and Johnston, R. B. (1984) *J. Exp. Med.* **160,** 1656–1671.
24. Arumoa, O. I., and Halliwell, B. (1987) *Biochem. J.* **241,** 273–278.
25. Buettner, G. R. (1987) *J. Biol. Chem.* **262,** 11,995–11,998.
26. Saito, M., Morehouse, L., and Aust, S. (1986) *J. Free Rad. Biol. Med.* **2,** 99–105.
27. Pollack, Vanderhoft, and Lasky, F. (1977) *Biochim. Biophsy. Acta.* **497,** 481–487.
28. Bostian, K. A., Blackburn, B. S., Wannemacher, R. W., McGann, V. G., and Biesel, W. R. (1976) *J. Lab. Clin. Med.* **87,** 577–585.
29. Edlow, D. W., and Sheldon, W. H. (1971) *Proc. Soc. Exp. Med.* **137,** 1378–1382.
30. Chance, B., Clark, B. J., Nioka, S., Subramanian, H., Maris, J. M., Argo, V. Z., and Bode, H. (1985) *Circulation* **72**(SIV), 103–110.
31. Etherington, D., Pugh, D., and Silver, I. (1981) *Acta Biol. Med. Germ.* **40,** 1625–1636.