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Mechanisms of Oxidation of $K_4Fe(CN)_6$ by Hypochlorous Acid and Catalytic Activation by Azide, Bromide, and Iodide

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Summary. HOCl reacts with $Fe(CN)_6^{4-}$ to generate an intermediate, presumably $FeCl(CN)_6^{3-}$, which exhibits a weak absorption around 282 nm and decays simultaneously with the formation of $Fe(CN)_6^{3-}$. When decreasing the HOCl/$Fe(CN)_6^{4-}$ concentration ratio from $R > 1$ to $R < 1$, a drastic change in the kinetics of the oxidation is observed. Depending on R, the intermediate obviously oxidizes either $Fe(CN)_6^{4-}$ or HOCl. At $R \gg 1$, a further intermediate appears which also precedes the oxidation and absorbs strongly around 360 nm. The intermediates detected may represent reactive high oxidation states of iron (Fe(IV) and Fe(VI)). HOCl induced oxidation of $Fe(CN)_6^{4-}$ is activated catalytically by Br^-, I^-, and N_3^-, obviously due to generation of stronger oxidants (HOBr, HOI, and ClN_3), but oxidation is efficiently inhibited by CN^- and NCS^-.

Keywords. Hypochlorous acid; Ferrocyanide oxidation by HOCl; *Fenton* reaction; Halide radicals.

Mechanismen der Oxidation von $K_4Fe(CN)_6$ durch Hypochlorsäure und katalytische Aktivierung durch Azid, Bromid und Iodid

Zusammenfassung. HOCl reagiert mit $Fe(CN)_6^{4-}$ unter Bildung eines Intermediats, vermutlich $FeCl(CN)_6^{3-}$, das bei 282 nm eine schwache Absorption aufweist und parallel zum Erscheinen von $Fe(CN)_6^{3-}$ verschwindet. Man beobachtet eine drastische Änderung in der Oxidationskinetik, wenn das HOCl/$Fe(CN)_6^{4-}$ Konzentrationsverhältnis von $R > 1$ zu $R < 1$ verändert wird. In Abhängigkeit von R wird offenbar entweder $Fe(CN)_6^{4-}$ oder HOCl durch das Intermediat oxidiert. Für $R \gg 1$ erscheint ein weiteres Intermediat mit einer starken Absorptionsbande bei 360 nm, das ebenfalls der Oxidation vorangeht. Bei den beobachteten Intermediaten handelt es sich vermutlich um reaktive höhere Oxidationsstufen des Eisens (Fe(IV) und Fe(VI)). Die HOCl-induzierte Oxidation von $Fe(CN)_6^{4-}$ wird einerseits durch Br^-, I^- und N_3^- katalytisch aktiviert (offenbar infolge der Bildung stärkerer Oxidantien wie HOBr, HOI und ClN_3), andererseits durch CN^- und NCS^- effektiv inhibiert.

Introduction

Reactions of hypochlorous acid (HOCl, $pK_a = 7.6$) with biological substrates have recently received increasing attention. HOCl is generated by activated polymorphonuclear leukocytes and is considered to play a major role in immune defense [1, 2]. On the other hand, it may cause tissue damage in certain inflammatory diseases such as rheumatoid arthritis [3]. HOCl tends to react by chlorination, for instance with amino groups [4, 5] and with unsaturated fatty acids [6, 7], and there is evidence that it can lead to the generation of free radicals in reactions with iron and copper complexes [8–10].

As a model for the redox interaction of HOCl with Fe(II), the oxidation of $Fe(CN)_6^{4-}$ has been investigated by several authors [8, 9, 10b, 11]. Since benzoate hydroxylation could be detected in this system, it was concluded that single electron transfer between Fe(II) and HOCl generates $^{\cdot}OH$ radicals *via* a *Fenton* type reaction [8]:

$$Fe(CN)_6^{4-} + HOCl \longrightarrow Fe(CN)_6^{3-} + {}^{\cdot}OH + Cl^- \qquad (1)$$

The $^{\cdot}OH$ radical, however, is obviously not the only reactive species resulting from the reaction of HOCl with Fe(II) complexes. As an alternative, it was suggested that the reaction might also lead to high oxidation states of iron, such as Fe(IV) [9]. Under the condition $[HOCl] \gg [Fe(CN)_6^{4-}]$, a rate constant of $k_1 = 220\,M^{-1}s^{-1}$ (at *pH* 5) was estimated, decreasing with the pK_a of HOCl to about 120 and 15 $M^{-1}s^{-1}$ at *pH* 7 and 9, respectively. Previously it has been noted that the rate constant of the HOCl induced oxidation of $Fe(CN)_6^{4-}$ at a given *pH* decreases when going to $[HOCl] < [Fe(CN)_6^{4-}]$. To explain this result, a reversible reaction with generation of $Cl^{\cdot}$ radicals has been proposed [11]:

$$Fe(CN)_6^{4-} + HOCl \rightleftharpoons Fe(CN)_6^{3-} + Cl^{\cdot} + OH^- \qquad (2)$$

However, since $Cl^{\cdot}$ is a very powerful one-electron oxidant ($E^\circ = 2.6\,V$ [12]), reduction of Fe(III) *via* the reverse reaction of Eq. (2) is highly unfeasible. At $[HOCl] \ll [Fe(CN)_6^{4-}]$, a rate constant of $2.1\,M^{-1}s^{-1}$ has recently been obtained at *pH* 6.9 [10b]; this is in strong contrast to the value of $120\,M^{-1}s^{-1}$ at $[HOCl] \gg [Fe(CN)_6^{4-}]$ (see above). With $Fe(CN)_6^{4-}$ in large excess over HOCl, the following mechanism has been tentatively proposed:

$$Fe(CN)_6^{4-} + HOCl \longrightarrow Fe^{IV}Cl(CN)_6^{3-} + OH^- \qquad (3)$$

$$Fe^{IV}Cl(CN)_6^{3-} + Fe(CN)_6^{4-} \longrightarrow 2Fe(CN)_6^{3-} + Cl^- \qquad (4)$$

HOCl induced oxidation of $Fe(CN)_6^{4-}$ has been applied as a probe for investigating the stoichiometry of the interaction of HOCl with biological substrates (*B*) and has furthermore been employed to obtain information about possible reactive products formed by interactions between *B* and HOCl [10b]. Particularly in this context it appeared desirable to elucidate in further detail the mechanism of HOCl induced oxidation of $Fe(CN)_6^{4-}$. The results of the present study reveal that the oxidation of $Fe(CN)_6^{4-}$ indeed proceeds *via* intermediates and that it is inhibited by cyanide and thiocyanate, but activated catalytically by azide, bromide, and iodide.

Results and Discussion

Oxidation of $Fe(CN)_6^{4-}$ at various $HOCl/Fe(CN)_6^{4-}$ concentration ratios R

The graphs of Fig. 1, obtained at $R = 0.5$, show that the slow formation of $Fe(CN)_6^{3-}$ (panel a) is accompanied by an intermediate which is formed within 15 s

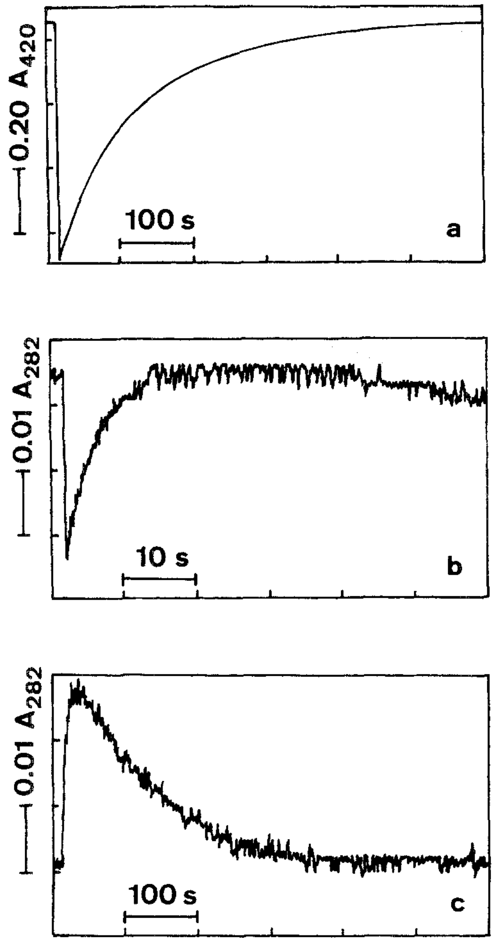

Fig. 1. Stopped-flow time profiles of absorbance changes upon reaction of 0.5 m*M* HOCl with 1 m*M* $K_4Fe(CN)_6$ in 10 m*M* phosphate buffer of *pH* 7.0; panel **a**: build-up of $Fe(CN)_6^{3-}$ absorption at 420 nm; panels **b** and **c**: formation and decay, respectively, of an intermediate absorbing at 282 nm

(panel b) and decays simultaneously with the build-up of $Fe(CN)_6^{3-}$ (panel c). The weak absorption of the intermediate overlaps with the strong absorbance change by the $Fe(CN)_6^{4-} \rightarrow Fe(CN)_6^{3-}$ transition and is therefore detectable only at the isosbestic point of this transition (282 nm). The $HOCl/ClO^-$ couple also absorbs around 282 nm, but HOCl removal would have led to a negative absorbance change.

At $R > 5$, the fast build-up of $Fe(CN)_6^{3-}$ (Fig. 2a) is accompanied by the formation and decay of a new intermediate (Fig. 2b) with an absorption around 360 nm which is much stronger than that due to the $Fe(CN)_6^{4-} \rightarrow Fe(CN)_6^{3-}$ transition as defined by the difference between start and end of trace b. At $R < 3$, the "360 nm intermediate" is only indicated by a hump in the time profile, thus making obvious that its formation requires HOCl in large excess. This species absorbs to above 400 nm and causes a distinct distortion even of 420 nm time profiles (Fig. 2a) used to monitor $Fe(CN)_6^{3-}$ formation. It was confirmed that the apparent rate constant of HOCl induced oxidation of $Fe(CN)_6^{4-}$ is about 60 times higher at $R \gg 1$ than at $R \ll 1$ (see Introduction), but in view of the distortion of time

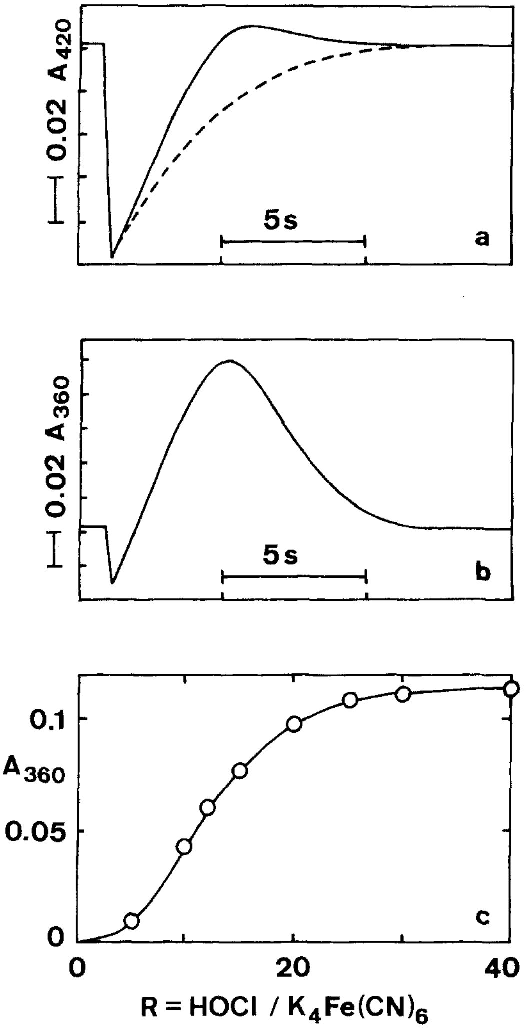

Fig. 2. Reaction of 0.5 to 4 m*M* HOCl with 0.1 m*M* $K_4Fe(CN)_6$ in 10 m*M* phosphate buffer, investigated by the stopped-flow technique; panel **a**: build-up of $Fe(CN)_6^{3-}$ absorption at 420 nm ($R = 20$, $pH = 7$); panel **b**: formation and decay of an intermediate at 360 nm ($R = 20$, $pH = 7$); panel **c**: peak absorbance at 360 nm, from time profiles as in panel b, as a function of the HOCl/$Fe(CN)_6^{4-}$ concentration ratio R ($pH \approx 6$); the dotted curve in panel a is a constructed time profile of $Fe(CN)_6^{3-}$ formation, allowing for distortion by the intermediate

profiles it appears ambiguous to give accurate numbers at $R \gg 1$. The yield of the "360 nm intermediate" (at *pH* 6) increases sigmoidally with R and reaches saturation first at $R > 30$ (Fig. 2c), suggesting that a chemical equilibrium is involved in its formation. The "360 nm intermediate" cannot be detected at $pH > pK_a$ (HOCl); this may indicate that ClO^- is unable to generate this species.

Semi-logarithmic plots of 420 nm time profiles for the HOCl induced oxidation of $Fe(CN)_6^{4-}$ at $R = 10$ to 2.5 show the character of non-exponential shoulder curves (Fig. 3), and surprisingly the oxidation becomes slower on increasing $[Fe(CN)_6^{4-}]$ at constant [HOCl]. The latter result is consistent with previous observations [11]. The shoulder in the time profiles indeed suggests that $Fe(CN)_6^{3-}$ is not formed directly by electron transfer from $Fe(CN)_6^{4-}$ to HOCl, as in reaction (1), but rather *via* precursors.

At $R \ll 1$, the stoichiometry has previously been found to be two $Fe(CN)_6^{3-}$ per HOCl [10b]; thus, this system provides a convenient means for quantitative determination of HOCl. In attempts to estimate the stoichiometry of oxidation at

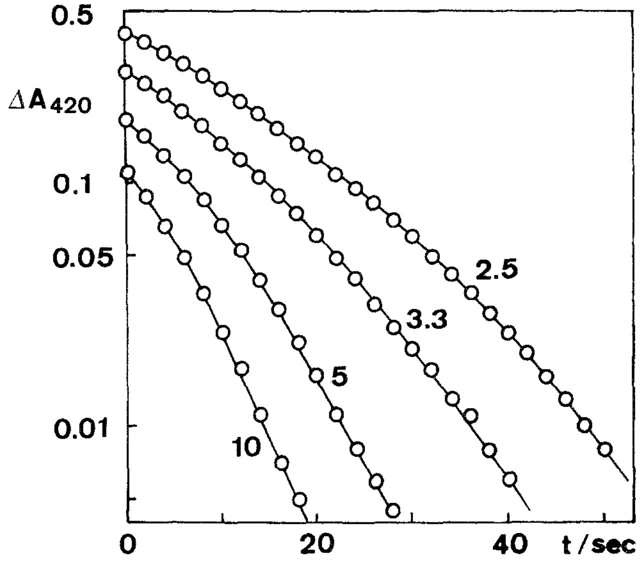

Fig. 3. Semi-logarithmic plots of time profiles of absorbance changes at 420 nm due to $Fe(CN)_6^{3-}$ formation upon reaction of 1 m*M* HOCl with 100, 200, 300, and 400 μ*M* $K_4Fe(CN)_6$, respectively, in 10 m*M* phosphate buffer of *pH* 7.0; the curves are labelled with the initial HOCl/$Fe(CN)_6^{4-}$ concentration ratio *R*

$R \gg 1$ we have reacted 0.2 m*M* $Fe(CN)_6^{4-}$ with 1.2 m*M* HOCl (solution A). Then, an excess of $Fe(CN)_6^{4-}$ was added to solution A (final $R = 0.06$) to determine the remaining HOCl. Surprisingly, the oxidation was faster when HOCl had been treated with $Fe(CN)_6^{4-}$ in solution A, as compared to a parallel investigation without such pretreatment. Furthermore, the oxidizing capacity of remaining HOCl was gradually lost when solution A was allowed to rest in the dark for about 30 min. This result suggests that the reaction of an excess of HOCl with $Fe(CN)_6^{4-}$ leads to the generation of products which are more reactive than HOCl itself and even capable of removing HOCl. Despite these complications, it was possible to estimate by extrapolation that about one HOCl was removed per $Fe(CN)_6^{4-}$ in solution A.

When unbuffered solutions of 2 m*M* $K_4Fe(CN)_6$ and 0.2 m*M* HOCl, both at *pH* 6.2, were mixed, a drastic increase of *pH* to 8.4 was obtained. On the other hand, no change in *pH* occured upon mixing of unbuffered solutions of 2 m*M* HOCl and 0.2 m*M* $K_4Fe(CN)_6$ (both at *pH* 6.2). This clearly reveals that there is a drastic change in the mechanism of interaction between $Fe(CN)_6^{4-}$ and HOCl when going from $R = 0.1$ to $R = 10$.

HOCl Induced oxidation of $Fe(CN)_6^{4-}$ in the presence of halides and pseudohalides

As shown in Fig. 4a, azide very efficiently accelerates the oxidation of $Fe(CN)_6^{4-}$ by HOCl at $[Fe(CN)_6^{4-}] \gg [HOCl] \gg [N_3^-]$ without changing the final yield of

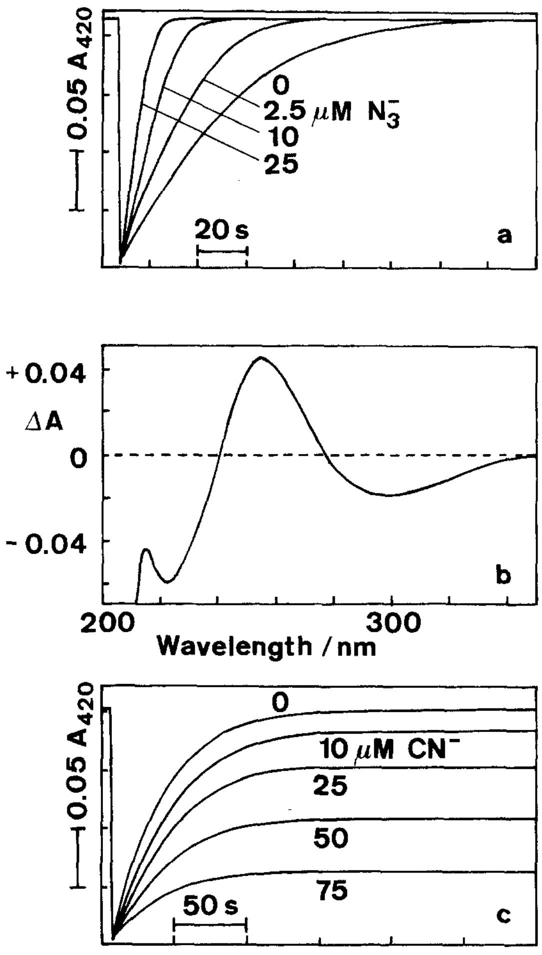

Fig. 4. Stopped-flow time profiles of absorbance changes at 420 nm upon reaction of 100 μ*M* HOCl with 5 m*M* $K_4Fe(CN)_6$ in presence of various concentrations of NaN_3 (panel **a**) or KCN (panel **c**); concentrations are indicated; panel **b** shows an absorbance difference spectrum about 1 min after reaction of 250 μ*M* HOCl with 250 μ*M* NaN_3, obtained with a two compartment tandem cell; solutions were buffered at *pH* 7.0 with 10 m*M* phosphate

$Fe(CN)_6^{3-}$; thus, N_3^- obviously acts catalytically. Cyanide, on the other hand, inhibits HOCl induced oxidation of $Fe(CN)_6^{4-}$ (Fig. 4c).

In absence of $Fe(CN)_6^{4-}$, the interaction between HOCl and N_3^- leads to immediate absorption changes (Fig. 4b), indicating a loss of HOCl/ClO^- absorption (290 nm) and formation of a fairly stable product absorbing around 260 nm. This reaction was too fast to be resolved with our stopped-flow system ($k > 10^5\, M^{-1}s^{-1}$ at *pH* 7). No reaction of ClO^- with N_3^- was detectable at *pH* 10. Time profiles as in Fig. 4a were also obtained when HOCl was reacted with N_3^- prior to mixing with $Fe(CN)_6^{4-}$, but the oxidative capacity of the HOCl/N_3^- solution slowly vanished after 30 min. At $R = 6$, formation of the "360 nm intermediate" was suppressed when N_3^- was added in excess over HOCl; the rapid oxidation of $Fe(CN)_6^{4-}$, however, was not inhibited. These results reveal that the reaction product of HOCl with N_3^- is a more powerful oxidant than HOCl, but oxidation obviously proceeds by a different mechanism.* Also, HOCl induced oxidation of a

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* At $[Fe(CN)_6^{4-}] \gg [N_3^-] \gg [HOCl]$, the rate of oxidation was first order in $[Fe(CN)_6^{4-}]$ but independent of $[N_3^-]$: $k_{obs} = 42\, M^{-1}s^{-1}$ (to be assigned later).

DNA-Cu(I) complex [10a] was found to be speeded up dramatically upon addition of azide.

HOCl induced oxidation of $Fe(CN)_6^{4-}$ can also be activated by Br^- and I^- (Fig. 5), no change in the final yield of $Fe(CN)_6^{3-}$ being observable as in the case of N_3^-. The activating effect increases in the order $Br^- < N_3^- \ll I^-$. Few n*M* iodide actually suffice to activate oxidation of m*M* quantities of $Fe(CN)_6^{4-}$; thus, we are clearly again dealing with a catalytic reaction. HOCl rapidly reacts with Br^- and I^- to generate HOBr and HOI, respectively [13a], and it appears resonable to assume that these species are the reactive chain carriers in the catalytic process (X^- = halide):

$$HOCl + X^- \longrightarrow Cl^- + HOX \tag{5}$$

$$Fe(CN)_6^{4-} + HOX \longrightarrow Fe(CN)_6^{3-} + {}^{\cdot}OH + X^- \tag{6a}$$

$$\longrightarrow Fe(CN)_6^{3-} + OH^- + X^{\cdot} \tag{6b}$$

The transient absorption resulting from interaction of HOCl with $Fe(CN)_6^{4-}$ vanishes upon adding increasing concentrations of Br^- or I^-; thus, oxidation by HOCl *via* intermediates is obviously replaced by oxidation *via* direct electron transfer in the presence of HOBr or HOI (Eq. (6)). The second oxidation step is accomplished by the ${}^{\cdot}OH$ or $X^{\cdot}$ radical.

In the case of catalysis by iodide, the build-up of $Fe(CN)_6^{3-}$ is a nearly linear function of time under appropriate conditions (Fig. 5a). This is reasonable because reaction (5) is very fast ($k_5 = 3.3 \times 10^8 M^{-1}s^{-1}$, [14]). Therefore, the rate-determining reaction (6), which leads to recycling of I^- and HOI, will virtually

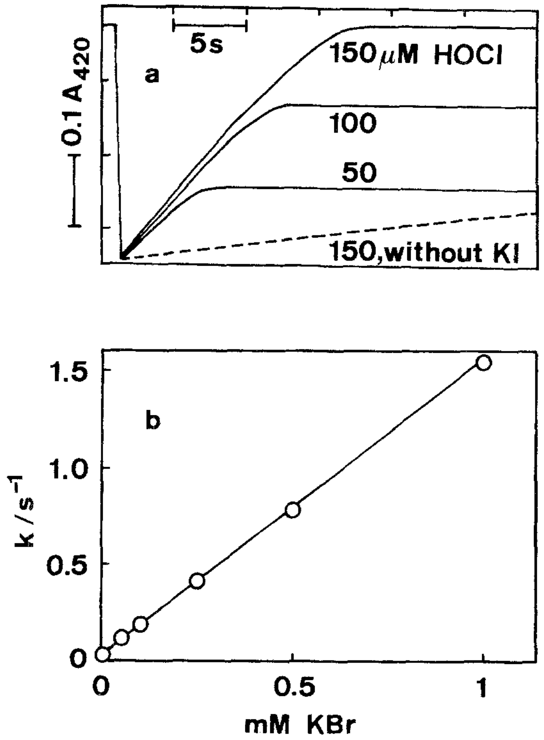

Fig. 5. Activation of the oxidation of $Fe(CN)_6^{4-}$ (5 m*M*) by HOCl in 10 m*M* phosphate buffer of *pH* 7 by KBr and KI, respectively; panel **a**: stopped-flow time profiles of oxidation by 50 to 150 μ*M* HOCl in presence of 10 n*M* KI (dotted curve: oxidation by 150 μ*M* HOCl in absence of KI); panel **b**: rate of oxidation by 100 μ*M* HOCl as a function of KBr concentration

proceed at a constant concentration of [HOI] $\approx$ $[I^-]_o$ until HOCl is depleted. The HOCl concentration merely determines the final level of oxidation and the total reaction period, but not the rate (Fig. 5a). The rate of oxidation, at the conditions applied, was linear in $[Fe(CN)_6^{4-}]$ and $[I^-]$ with a rate constant of k_6 (HOI) $\approx 4.4 \times 10^5 M^{-1}s^{-1}$. Time profiles as in Fig. 5a were obtained also when HOCl was reacted with I^- shortly before addition of $Fe(CN)_6^{4-}$, but the activating effect of I^- vanished within 10 min, obviously due to slow oxidation of HOI to IO_3^- [13a].

In the case of bromide, the rate of oxidation was found to increase linearly with $[Br^-]$ both at $[Br^-] < [HOCl]$ and $[Br^-] > [HOCl]$ (Fig. 5b), and time profiles remain exponential in contrast to the case of I^-. The implication seems to be that with Br^- reaction (5) is rate-determining. When HOBr was generated *via* reaction (5) prior to addition of $Fe(CN)_6^{4-}$ using concentrations as in Fig. 5b, reaction (6) was indeed too fast to be resolved. Reduction of the concentrations of the components permitted a rough estimate of the rate constant: $k_6(HOBr) \approx 3\times 10^4 M^{-1}s^{-1}$. Due to the fast recycling of Br^- *via* reaction (6), the concentration of Br^- remains constant as the reaction cycle proceeds, and conceivably the rate must increase linearly with $[Br^-]$ even at $[Br^-] < [HOCl]$. From the slope in Fig. 5b, a rate constant of $k_5(Br^-) = 1.56 \times 10^3 M^{-1}s^{-1}$ can be estimated, in reasonable agreement with $2.95 \times 10^3 M^{-1}s^{-1}$ previously obtained by extrapolation of rate measurements in the *pH* range of 10 to 14 [13a].

HOCl induced oxidation of $Fe(CN)_6^{4-}$ was not affected by NCO^-, but was inhibited by CN^- (Fig. 4c). Cyanide obviously acts as HOCl scavenger by instantaneous generation of NCO^- [13a]. The time profiles in Fig. 4c actually show the oxidation of $Fe(CN)_6^{4-}$ by residual HOCl remaining after the initial very fast removal by CN^-, and the loss of oxidative capacity is consistent with removal of one HOCl per CN^-. A variety of substrates (ascorbate, thiols, *NADH* and *TRIS*) have previously been found to act as HOCl scavenger, too, without generating reactive products capable of oxidizing $Fe(CN)_6^{4-}$ [10b].

Thiocyanate was found to be an even more efficient HOCl scavenger than CN^-. The remarkable difference was that, in an experiment as in Fig. 4c, four HOCl molecules were removed per NCS^-. This stoichiometry corresponds to that of NCS^- oxidation by hypobromite: $4BrO^- + NCS^- + H_2O \rightarrow 4Br^- + NCO^- + H_2SO_4$ [13b]. It has to be mentioned in this context that sulfhydryls and disulfides also can scavenge up to four HOCl by consecutive sulfoxidations [10b].

We have noted that HOCl induced oxidation of $Fe(CN)_6^{4-}$ can be activated also by nitrite, though in this case not by a catalytic process. It is commonly assumed that HOCl reacts with NO_2^- to generate nitrate [13a]. Our observation indicates that a reactive intermediate, possibly peroxynitrite ($ONOO^-$) or nitryl chloride ($ClNO_2$), is formed in this reaction.

Mechanism of HOCl induced oxidation of $Fe(CN)_6^{4-}$

The present results confirm that the kinetics of HOCl induced oxidation of $Fe(CN)_6^{4-}$ strongly depend on the concentration ratio *R*, and in particular they reveal that intermediates are formed which apparently play an active role in the oxidative process. The following reaction scheme is proposed to explain these results:

$$2\mathrm{Fe}(\mathrm{CN})_6^{3-} + \mathrm{Cl}^- \tag{4}$$

$$\uparrow \mathrm{Fe}(\mathrm{CN})_6^{4-}$$

$$\mathrm{Fe}(\mathrm{CN})_6^{4-} + \mathrm{HOCl} \rightleftarrows \mathrm{FeCl}(\mathrm{CN})_6^{3-} + \mathrm{OH}^- \tag{3}$$

$$\downarrow \mathrm{HOCl}$$

$$\mathrm{Fe}(\mathrm{CN})_6^{3-} + \mathrm{HCl} + {}^{\cdot}\mathrm{OCl} \tag{7a}$$

Further reaction pathways are also envisaged:

$$\mathrm{FeCl}(\mathrm{CN})_6^{3-} + \mathrm{HOCl} \rightarrow \mathrm{Fe}(\mathrm{CN})_6^{3-} + \mathrm{Cl}_2 + {}^{\cdot}\mathrm{OH} \tag{7b}$$

$$\mathrm{FeCl}(\mathrm{CN})_6^{3-} + \mathrm{HOCl} \rightleftarrows \mathrm{FeCl}_2(\mathrm{CN})_6^{2-} + \mathrm{OH}^- \tag{7c}$$

The fast build-up of the "282 nm intermediate" (Fig. 1b) can be explained by reaction (3). Reaction (4) is apparently the rate-determining step at $R \ll 1$. When going to $R \gg 1$, reaction (3) removes $\mathrm{Fe}(\mathrm{CN})_6^{4-}$; as a consequence, reaction (4) becomes unfeasible and is replaced by the faster reaction (7a) and/or reactions (7b) and (7c) (discussed below). The previously mentioned discrepancy in the rate constants for the HOCl induced oxidation of $\mathrm{Fe}(\mathrm{CN})_6^{4-}$ can be explained if the values obtained at $R \ll 1$, and $R \gg 1$ (see Introduction) are assigned to k_4 and k_7, respectively. There are arguments are in favour of reaction (3) involving chlorination of the iron complex (see below), but it cannot be excluded that the intermediate detected is a ferryl species, $\mathrm{O} = \mathrm{Fe}(\mathrm{CN})_6^{4-}$, which mediates the oxidation of either $\mathrm{Fe}(\mathrm{CN})_6^{4-}$ or HOCl depending on R.

A further argument in favour of the above scheme is the remarkable difference in the *pH* change induced by mixing the reagents in unbuffered solutions at $R \ll 1$ and $R \gg 1$, respectively. Consistent with our observations, the above scheme predicts no change of *pH* at $R \gg 1$ where the reaction sequence (3)–(7a) prevails, and an increase of *pH* at $R \ll 1$, where the reaction sequence (3)–(4) dominates.

The surprising decrease in the rate of oxidtion upon increasing the $\mathrm{Fe}(\mathrm{CN})_6^{4-}$ concentration as well as the curvature in the semi-logarithmic time profiles at $R = 10$ to 2.5 (Fig. 3) are also consistent with the sequence of reactions (3) and (7a): the higher the $\mathrm{Fe}(\mathrm{CN})_6^{4-}$ concentration, the lower is the HOCl concentration remaining after reaction (3) for the subsequent rate-determining oxidative reaction (7a). The $\mathrm{ClO}^{\cdot}$ radical proposed to be generated in reaction (7a) is a very strong one-electron oxidant [15] and should conceivably be capable of oxidizing a second $\mathrm{Fe}(\mathrm{CN})_6^{4-}$:

$$\mathrm{Fe}(\mathrm{CN})_6^{4-} + \mathrm{ClO}^{\cdot} \rightarrow \mathrm{Fe}(\mathrm{CN})_6^{3-} + \mathrm{ClO}^- \tag{8}$$

Regeneration of HOCl by reaction (8) promotes the oxidation as the reaction proceeds. At $R \gg 1$, an interaction of $\mathrm{ClO}^{\cdot}$ with the product of reaction (3) must also be envisaged, for instance:

$$\mathrm{FeCl(CN)_6^{3-} + ClO^{\cdot} \rightarrow Fe(CN)_6^{3-} + Cl_2O} \qquad (9)$$

The progressive loss of HOCl after oxidation of $\mathrm{Fe(CN)_6^{4-}}$ at $R > 1$ may be due to interactions between $\mathrm{Cl_2O}$ and HOCl, but at present no possible reactions can be specified.

Reaction (7a) does not explain the generation of $\mathrm{^{\cdot}OH}$ radicals as indicated in previous experiments [8] and depicted by reaction (1). A possible route to the generation of $\mathrm{^{\cdot}OH}$ radicals could be reaction (7b). The $\mathrm{^{\cdot}OH}$ radical would react with HOCl to generate $\mathrm{ClO^{\cdot}}$, and $\mathrm{Cl_2}$ would hydrolyze to HCl and HOCl, thus the overall outcome of reaction (7b) being the same as that of reaction (7a). $\mathrm{^{\cdot}OH}$ radicals are not the only reactive species resulting from reactions of HOCl with $\mathrm{Fe(CN)_6^{4-}}$ [8, 9]; it is proposed that both reactions (7a) and (7b) are relevant. It might be assumed that the $\mathrm{FeCl(CN)_6^{3-}}$ species also decays directly to $\mathrm{Fe(CN)_6^{3-}}$ by elimination of $\mathrm{Cl^{\cdot}}$ which is in equilibrium with $\mathrm{\cdot OH}$ radicals [9]. However, $\mathrm{Cl^{\cdot}}$ or $\mathrm{^{\cdot}OH}$ radicals thus formed would immediately react with HOCl at $R \gg 1$ or with $\mathrm{Fe(CN)_6^{4-}}$ at $R \ll 1$, and the observed change in the reaction kinetics would not be explainable.

Generation of the reactive intermediate $\mathrm{FeCl(CN)_6^{3-}}$ was previously proposed on the basis that nucleotides like inosine and thymidine, after chlorination by HOCl, are capable of oxidizing $\mathrm{Fe(CN)_6^{4-}}$ at a slower rate than HOCl but with the same stoichiometry [10b]. Obviously, HOCl can be replaced in reaction (3) by other chlorinating compounds. The $\mathrm{FeCl(CN)_6^{3-}}$ species was suggested to contain a high oxidation state of iron (Fe(IV)). Since HOCl reacts with cyanide to generate $\mathrm{NCO^-}$ [13a], it might be suggested that reaction (3) alternatively leads to a modification of the $\mathrm{CN^-}$ ligands of iron. There is, however, no evidence for $\mathrm{CN^-}$ ligand modification by HOCl in the case of $\mathrm{Fe(CN)_6^{3-}}$.

The "360 nm intermediate" appears only when HOCl is in large excess over $\mathrm{Fe(CN)_6^{4-}}$ (Fig. 2b, c). This suggests that $\mathrm{Fe(CN)_6^{4-}}$ is capable of accommodating more than one Cl atom, as depicted by reaction (7c). The proposed $\mathrm{FeCl_2(CN)_6^{2-}}$ species may contain a reactive Fe(VI) oxidation state reacting with HOCl (at $R \gg 1$) for instance *via* reaction (10):

$$\mathrm{FeCl_2(CN)_6^{2-} + HOCl \rightarrow Fe(CN)_6^{3-} + H^+ + Cl_2 + ClO\cdot} \qquad (10)$$

The sigmoidal concentration dependence of the "360 nm species" (Fig. 2c) indicates that reactions (3) and (7c) are equilibria. It is not possible, however, to evaluate equilibrium constants from Fig. 2c since the species is removed under reaction. Oxidation of $\mathrm{Fe(CN)_6^{4-}}$ vanishes in alkaline solution [8], and coincidently also formation of the "360 nm species". This coincidence indicates in my opinion that the reverse reactions of Eqs. (3) and (7c) in alkaline solution prevent formation of the reactive intermediates mediating the oxidation of $\mathrm{Fe(CN)_6^{4-}}$. An alternative view is that the $\mathrm{ClO^-}$ anion is unable to interact with $\mathrm{Fe(CN)_6^{4-}}$ [9].

It should be mentioned that the $\mathrm{ClO_2^{\cdot}}$ radical also exhibits an absorption around 360 nm [17]. It is difficult, however, to design mechanisms of formation of $\mathrm{ClO_2^{\cdot}}$ in the system investigated. In particular, the $\mathrm{ClO_2^{\cdot}}$ radical would react immediately with $\mathrm{Fe(CN)_6^{4-}}$ ($k = 7.4 \times 10^7\, M^{-1}\mathrm{s}^{-1}$, [16]) and persist after removal of $\mathrm{Fe(CN)_6^{4-}}$ as it is a stable radical.

The effect of halides and pseudohalides

Catalytic activation of HOCl induced oxidation of $Fe(CN)_6^{4-}$ by Br^- and I^- is readily explained by the transformation of HOCl into HOBr and HOI (reaction (5)). Reaction (6a) directly regenerates the halide (X^-), but alternatively reaction (6b) might lead to formation of $X^{\cdot}$ radicals which at high halide concentrations equilibrate to the dihalogen radical anion ($X_2^{\cdot-}$). All these halide species are strong oxidants [16] which by reaction with $Fe(CN)_6^{4-}$ would regenerate X^-; therefore, both reaction pathways would yield the same overall reaction:

$$HOX + 2Fe(CN)_6^{4-} \rightarrow OH^- + X^- + 2Fe(CN)_6^{3-} \qquad (6)$$

Further experiments are required to enable distinction between reactions (6a) and (6b).

The catalytic activity of azide (Fig. 4a) is most likely due to the formation of chlorine azide [18] as reactive agent:

$$HOCl + N_3^- \rightleftarrows ClN_3 + OH^- \qquad (11)$$

$$Fe(CN)_6^{4-} + ClN_3 \rightarrow Fe(CN)_6^{3-} + Cl^- + N_3^{\cdot} \qquad (12)$$

$$Fe(CN)_6^{4-} + N_3^{\cdot} \rightarrow Fe(CN)_6^{3-} + N_3^- \qquad (13)$$

ClN_3 was found to be a chlorinating agent; it reacts for instance with the nucleotide *AMP* to produce the same absorbance change (though at a slower rate) as HOCl due to formation of *AMP*-chloroamine [10b]. We have no evidence, however, that ClN_3 induced oxidation of $Fe(CN)_6^{4-}$ proceeds *via* intermediates as in the case of HOCl. At $[N_3^-] > [HOCl]$, the oxidation rate became $[N_3^-]$ independent; the rate constant ($k_{obs} = 42\,M^{-1}s^{-1}$) can be assigned to reaction (12). Reaction (13), regenerating N_3^-, is much faster ($k_{13} = 3.4 \times 10^9\,M^{-1}s^{-1}$, [19]).

It is interesting to note that oxidation of $Fe(CN)_6^{4-}$ by HOCl can be accelerated also by catalytic amounts of Cu^{2+} and by nucleotides like *AMP* and *CMP* [10b]. Different mechanisms have to be adopted, though, to explain the activating effects of the various agents tested so far.

The results of the present study suggest that interactions of HOCl with biological substrates as well as the microbicidal activity of myeloperoxidase (the HOCl generating enzyme of polymorphonuclear leukocytes) may also be susceptible to halides and pseudohalides. N_3^- and CN^-, for instance, are known to inhibit myeloperoxidase [20]; the inhibitory effect, particularly of cyanide, may in part be due to scavenging of HOCl. A further investigation with the intention to reveal that halides and pseudohalides can severly modify the HOCl induced oxidation of protein and nucleic acid components is in progress.

Experimental

Sodium hypochlorite was diluted to the desired concentration from a 4% aqueous solution (Aldrich). The hypochlorite concentration was determined by its absorption, assuming an extinction coefficient of $\epsilon_{292}(ClO^-) = 385\,M^{-1}\,cm^{-1}$ at *pH* 10, *i. e.* the mean of literature data [21], and was controlled regularly also by monitoring the oxidation of $Fe(CN)_6^{4-}$ (2 per HOCl at $R \ll 1$) [10b] using ϵ_{420} ($Fe(CN)_6^{3-}$) $= 1050\,M^{-1}cm^{-1}$. Stock solutions of $K_4Fe(CN)_6$ (Merck), prepared daily, were deaerated by flushing with N_2 to avoid autooxidation. Potassium salts of halides and pseudo halides of highest purity commercially available were applied as received. Solutions were prepared with redistilled water for each experiment and protected against light-induced decomposition. All results refer to air-saturated phosphate-buffer solutions. It was controlled, however, that the reactions investigated are O_2 independent.

Stopped-flow experiments were performed with an SFA-12 Rapid-Kinetics-Accessory (HI-TECH Scientific Ltd.) coupled with a UV/Vis spectrophotometer (Shimadzu Corp.) using a 1 cm optical path mixing cell thermostatted at 22°C. Absorbance difference spectra, for instance due of reaction of HOCl with N_3^-, were obtained with a two-compartment tandem mixing cell of 2×4.375 mm optical path (Hellma). Generally, the concentrations after mixing of the components are stated. The present study was mainly restricted to neutral solutions because this is the range where the HOCl/ $Fe(CN)_6^{4-}$ system will be further applied as a reference to probe interactions of HOCl with biological substrates (see Introduction); it is obvious from previous investigations [8] that HOCl (not ClO^-) is the main reactivity entity.

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