Tin Tetrachloride Interaction With Human Hemoglobin

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Abstract. In the presence of tin tetrachloride, the rate constant for the rebinding of CO to the triliganded hemoglobin, l'_4, is much increased and the number of molecules participating in the CO ligation is decreasing. The pH drops, but a proton consumption process also takes place at the same time. The heme and its environment do not seem to change much, but the tin complexing to protein is very probable. The oxidation of hemoglobin in the form of either oxy or carbonmonoxy occurs with rather high rate.

Key words: Hemoglobin — Tin compounds — Hemoglobin-tin(IV) interaction — Laser photolysis

Introduction

Ligation and protonation process are related in the case of heme proteins, particularly of hemoglobin (Hb), through the interactions between the functional group and the protein. These processes have been extensively studied in order to understand the factors that control the binding and release of the physiological ligand. Different ions, anions or cations, can be bound in certain protein sites leading to changes of Hb conformation and thereby influencing Hb functional behaviour or moreover, favouring the electron transfer processes (Rifkind 1974, Winterbourn and Carrell 1977, Brittain 1980, Tomoda et al. 1981a, Tarburton and Metcalf 1986).

In several reports, the binding of CO to heme proteins has been monitored at different pH values (Schmeltzer et al. 1972, May and Mayer 1975, Braunstein et al. 1988, Serbanescu et al. 1989). Recent results gave evidence of the conformational changes in the heme pocket, accompanying the observed ligand binding enhancement at low pH (Han et al. 1990, Iben et al. 1991, Sage et al. 1992).

Starting from the oxidative and remarkable complexing properties of tin(IV)

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compounds (Hershberger et al. 1988, Rihter et al. 1990), we began to study *in vitro* the influence of tin tetrachloride upon Hb molecule, by spectroscopic means. To our knowledge, tin(IV) – Hb interactions were not described in the literature, although the use of some tin organics as drugs (Pascal 1963) or as photosensitizers (Rakestraw et al. 1990) is known. The results presented in this paper show not only an ordinary oxidation process, but conformational changes due to tin complexing and to protons.

Materials and Methods

Materials

Hemoglobin was extracted from fresh human normal blood by hemolysis and further purified by dialysis and chromatography. Details of the working procedure were given elsewhere (Serbanescu and Turcu 1987). The carbonmonoxy form (HbCO) was obtained by deoxygenating in vacuum the oxy form (HbO_2) and by CO bubbling up to saturation ([CO] = 1 mmol/l). Unbuffered solutions were used.

Total concentrations of HbO_2 and HbCO solutions were routinely determined by measuring the absorbance of the band at 568 and 576 nm respectively and using the corresponding extinction coefficients given by Van Kampen and Zijlstra (1983). The samples contained less than 5% metHb and deoxyHb forms.

Tin tetrachloride and aminoacids were from Merck. All the chemicals were of p.a. grade. Tin tetrachloride solutions were used immediately after preparation.

Laser photolysis

The experimental set-up and data processing for studying the gaseous ligand rebinding to heme after laser photolysis with a nanosecond pulse was previously described (Serbanescu and Frunza 1991). It consists in observing the ligand rebinding kinetics in the microsecond time range at Soret band (438 nm), using a memory Tektronix oscilloscope. Changes of total absorbance $\Delta A^{\text{total}}(t)$ were calculated in the microsecond time scale after the low level CO photolysis. Since the recombination process is biphasic, further data analysis resulted in the two rate constants corresponding to the fast and slow step, respectively.

At different moments (t_r) during the reaction of HbCO with tin tetrachloride, HbCO was photolysed and the CO rebinding was followed in the microsecond time range. Thus, the CO rebinding plays the monitor role for the functional state of HbCO during its reaction with tin compound. The above calculation procedure was repeated each time and the kinetic parameters of gaseous ligand rebinding were obtained as functions of the reaction time t_r.

Optical spectroscopy

The spectrum changes accompanying the reaction of HbO_2 or HbCO with tin tetrachloride solution were followed using a Specord UV-VIS (Jena) spectrometer, working either at a fixed repetition rate or as a function of time at a fixed wavelength. The reaction products were identified and their concentration was evaluated by using simultaneous equations (Van Kampen and Zijlstra 1983).

Initial molar concentration ratio between the reactants [Sn(IV)]/[heme] was in the range 1–5. Details were given in the Figure legends.

pH measurements

The pH values that we report here are those measured directly at different times after reactant mixing. It was used a Corning EEL-10 pH-meter equipped with a glass electrode which was calibrated with different standard buffers in the given pH range.

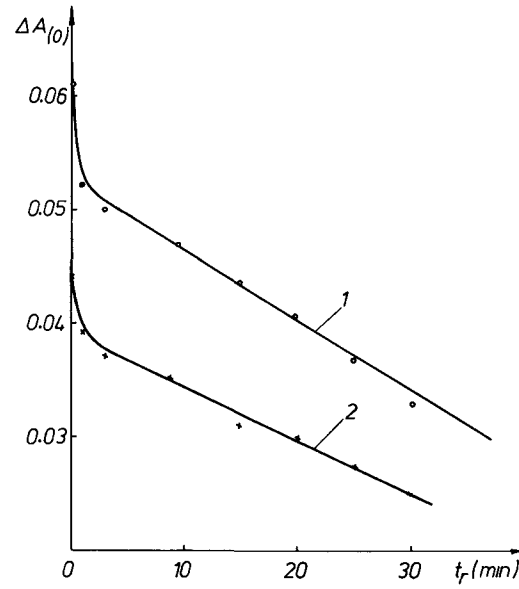

Figure 1. Dependence of the absorbance changes at zero photolysis time on the reaction time t_r, after the mixing of HbCO with $SnCl_4$. $[HbCO]_{t_r=0}$ = 70 μmol/l; [Sn(IV)]/[HbCO] = 1/1. 1) The total absorbance change ΔA^{total} (0); 2) The absorbance change for the faster step ΔA^{fast} (0).

Results

Laser photolysis

The total absorbance changes $\Delta A^{total}(0)$ due to HbCO photolysis measured at different moments during tin tetrachloride reaction with HbCO is plotted in Fig. 1, curve 1, as function of the reaction time t_r. The decreasing curve shows the diminution of HbCO molecules number rebinding CO after photolysis. The part of the total absorbance corresponding to the faster process $\Delta A^{fast}(0)$ has the same variation vs. reaction time as the total absorbance (Fig. 1, curve 2).

The fast process was previously ascribed to the binding of the CO molecule to the triply liganded Hb; thereby, the rate constant of this process corresponds to l'_4. The variation of l'_4 with the reaction time is given in Fig. 2. A marked

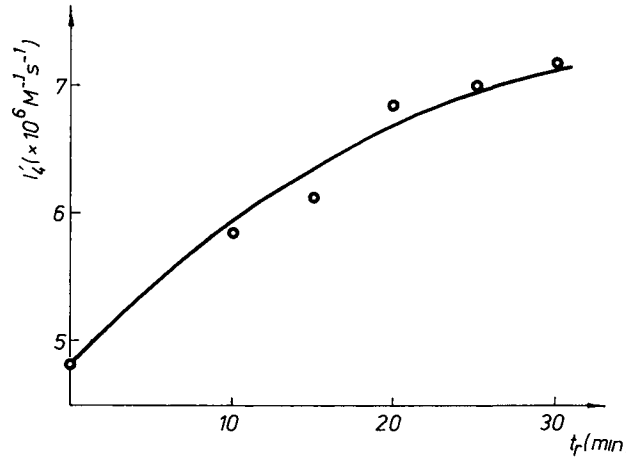

Figure 2. Variation of the l'_4 rate constant with the reaction time t_r, during HbCO interaction with $SnCl_4$. $[HbCO]_{t_r=0} = 70$ μmol/l; [Sn(IV)]/[HbCO] = 1/1.

enhancement of CO binding to Hb in the presence of tin tetrachloride is observed, the l'_4 increase being 85% after 30 min as compared to the value in the absence of tin compound.

The slower relaxation process as a pseudo-first-order reaction in CO excess has a R-like lifetime value of about 550 μs and a variation vs. reaction time much smaller than that of the faster process.

Optical spectroscopy

The most illustrative changes of the absorption spectra due to the action of tin tetrachloride upon HbO_2 and HbCO are given in Figs. 3*a* and 3*b*, respectively. Two peaks at 500 and 630 nm appeared after about 20 min, indicating the formation of high spin methemoglobin (metHb). The isosbestic points at 525 and 590 nm (Fig. 3*a*) and respectively at 519 and 585 nm (Fig. 3b) were preserved during this lapse of time, showing that no destruction of heme group took place. The final mixture contains about 30% unoxidized Hb, along with metHb.

Under the specified reaction conditions, the half life time was 82 s for HbO_2 and 96 s for HbCO, both these values being smaller than those estimated in other similar electron transfer processes (Serbanescu and Frunza 1991). For the reactant ratio 1/1, the spectra showed a reaction significantly slower than for 5/1 ratio.

The kinetic curves from Figs. 4*a* and 4*b* were obtained by recording the changes of α-band height as a function of time. The plot depends on the reactant ratio and on the catalase presence. The enzyme slows down to a great extent the reaction

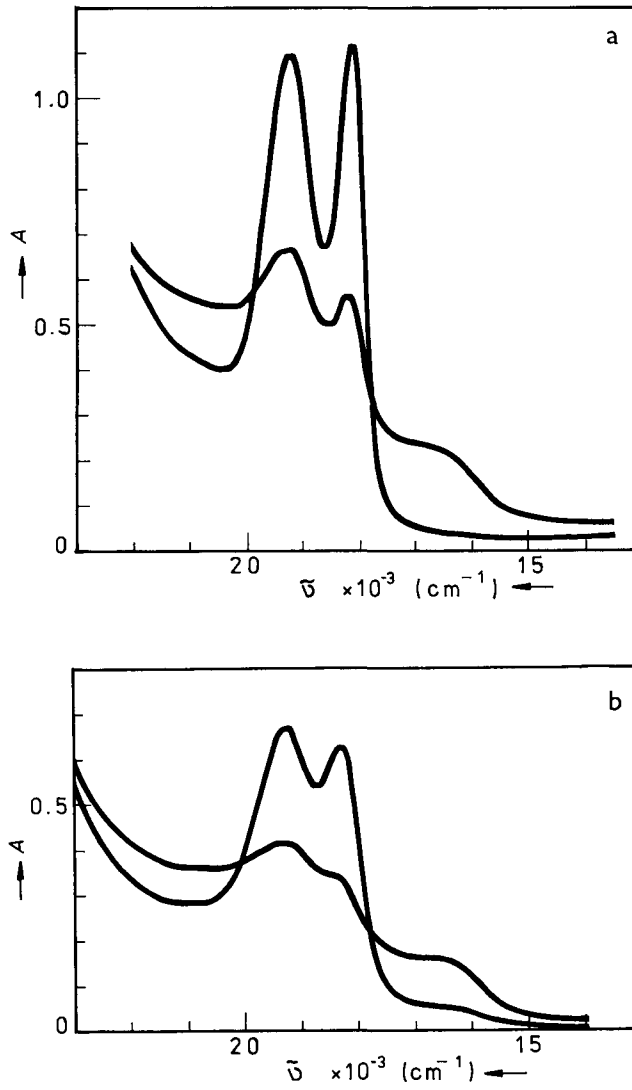

Figure 3. Changes in the absorption spectrum of: *a*) HbO_2; *b*) HbCO during the interaction with the tin compound. $[HbO_2]_{t_r=0}$ = 85 μmol/l; $[HbCO]_{t_r=0}$ = 40 μmol/l; [Sn(IV)]/[heme] = 5/1. The interval between the two recordings was 20 min and 17 min, respectively.

rate, showing the involvement of H_2O_2 as reaction intermediate. Spectra registered in the presence of catalase show the same features as those in Fig. 3 but taking place more slowly.

When tin tetrachloride is in excess, namely under pseudo-monomolecular reac-

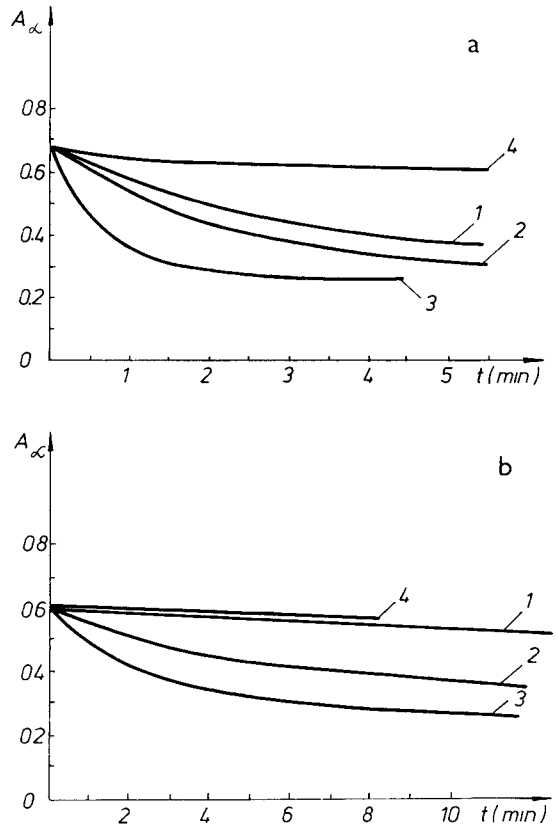

Figure 4. Variation of the α-band during the interaction between Sn(IV) and *a*) HbO_2; *b*) HbCO. *a*) $[HbO_2]_{t_1=0}$ = 80 μmol/l; [Sn(IV)]/[HbO_2] is: 1) 3/1; 2) 4/1; 3) 5/1; 4) 5/1 + catalase (1.3 μg/ml). *b*) $[HbCO]_{t_1=0}$ = 80 μmol/l; [Sn(IV)] /[HbCO] is 1) 3/1; 2) 4/1; 3) 5/1; 4) 5/1 + catalase (1.3 μg/ml).

tion conditions, the rate constants corresponding to HbO_2 and HbCO oxidation are respectively 0.08 s^{-1} and 0.06 s^{-1}, showing once more that HbO_2 is more rapidly oxidized than HbCO form.

The easy coordination of sulphide ion to Sn (Velazques et al. 1980) could indicate the cysteine residues in Hb molecules among the targets for tin tetrachloride action. Isolated cysteine indeed reacts with this tin compound since its UV absorption band around 220 nm is much increased in its presence. Instead, the spectrum has no peak at 240 nm where cysteine absorbs (Lehninger 1975) thus showing that cysteine oxidation has not taken place.

pH measurements

pH variation versus reaction time after the mixing of tin tetrachloride with HbO_2 or HbCO is given in Fig. 5. A sharp decrease up to ca. pH 4 can be observed in a few minutes for both Hb forms. The rate constant for this pH decrease is 0.116 s^{-1} in the case of HbO_2 and 0.082 s^{-1} for HbCO.

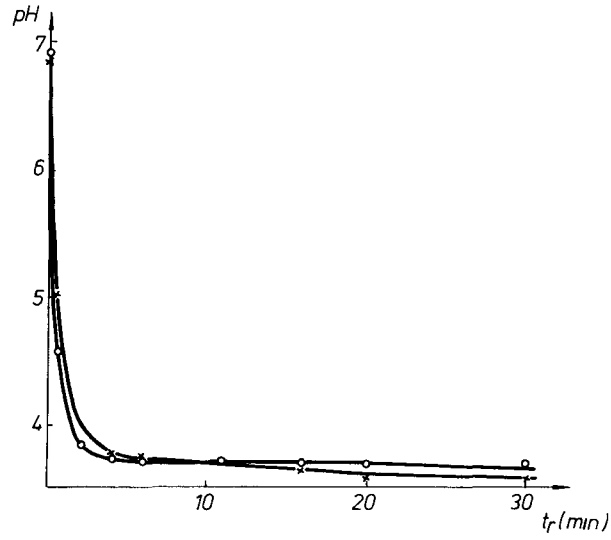

Figure 5. pH variation during HbCO (o – o – o) and HbO_2 (× – × – ×) interaction with the tin reagent. $[HbCO]_{t_r} = 70$ μmol/l; $[HbO_2]_{t_i=0} = 75$ μmol/l; [Sn(IV)]/[heme] = 2/1.

However, the pH decrease is much smaller than that assumed at the tetrachloride hydrolysis expense. Blank experiments of hydrolysis under the same concentration conditions led to pH 2 in a few seconds. Consequently, we have to suppose that a proton consumption process took place, as in the case of Hb oxidation under the influence of peroxidosulphate ions (Serbanescu and Frunza 1991). In the presence of tin compound, a proton gain of about $1H^+$/heme can be estimated within half of an hour.

Discussion

In the presence of tin tetrachloride, the kinetics of ligand binding is altered, the l'_4 is much increased as we have already shown. This rise of 85% cannot be merely explained on the basis of the pH decrease since previous experiments (Serbanescu et al. 1989) indicated an increase by only 19% upon the corresponding pH dropping. The pH induced increase has to be related to the conformational modifications of the R quaternary Hb state within the frame of the two states allosteric model. Thus, the iron-histidine bond can be ruptured at low pH and replaced by a bond with a weaker field ligand, the distal pocket becomes more open, accommodating a CO molecule in a more upright orientation (Han et al. 1990). An extra proton binding at histidine Nδ atom, already implied in a hydrogen bond with the backbone carbonyls has been proposed by Iben et al. (1991). The species which do

not contain any more the iron-histidine bond, have been recently put in evidence (Sage et al. 1992). On the other hand, not only proximal but distal phenomena have received attention in the last time (Levy et al. 1992) due to their role in heme cooperativity and in the mechanism of ligand binding.

In our case, such modifications can take place due to protons provided by tin tetrachloride hydrolysis.

Although optical spectroscopy generally offers information about the structure of heme and its environment, it is not enough to decide upon details as the substitution of the proximal histidine by another ligand or the excessive protonation of this residue. Thus, our spectra in the visible range have indicated only the formation of high spin metHb. They are also far from giving evidence of any relationship between the spin state and the allosteric equilibrium (Marden et al. 1991).

The most part of the observed l'_4 increase could be explained by the strain effects introduced by tin coordination to the protein, namely to different atoms carrying lone pair electrons. There were reported studies about the strain influence on the kinetics and equilibria of ligand binding to Hb (Geibel et al. 1978, Jayaraman et al. 1993); the linking of Zn ions leads to a 20 fold rise of the oxygen affinity of human Hb (Gilman and Brewer 1978, Gray and Dean 1982). The highest effects are to be expected when the deformation in the proximal imidazole direction is caused. We can speculate accordingly that such deformations have to be present in our case too.

Hb protein has many atoms of oxygen, nitrogen and sulphur, some of them are suitably positioned to coordinate but only few can close a chelating ring more or less rigid. There are several sites important from the coordination point of view as the SH group β-93 cysteine, the oxygens of β-94 asparagine, both in the neighbourhood of the proximal imidazole of β-92 histidine and accessible through the heme pocket, as well as other cysteine SH groups with lower accessibility. Tin bonding to SH group of isolated cysteine was spectrophotometrically proved.

In addition, an oxidation process for HbO_2 and HbCO was observed in the presence of tin compound. At first sight, iron oxidation could be easy put on the expense of tin reduction. It is known that the redox potential Sn^{4+}/Sn^{2+} has a value of 0.15 V under acidic pH conditions (Niac et al. 1984), whereas the corresponding one for iron is 0.168 V for HbO_2 at pH 6.3 (Santucci et al. 1984); also, it is known that chemically modified hemoglobins are more easily oxidized than the normal forms. However, we think that the observed oxidation seems to be related to an electron transfer rather to dioxygen than to tin under our conditions of conformational changes due to tin ligation. This oxidation is not simply an autoxidation process even running at low pH, since the observed rate is much higher than the autoxidation rate (Tomoda et al. 1981b).

The presence of H_2O_2 among the intermediates of oxidation process was presumed on the basis of catalase action, it could result by the following reaction

(Sutton et al 1976)

$$2O_2^- + 2H^+ \longrightarrow H_2O_2 + O_2$$

However, the other participants to the electron transfer remain still unknown

We concluded that the influence of tin tetrachloride upon Hb behaviour consists in the following facts tin is bound to the protein, one of the most probable binding sites being the SH group of β-93 cysteine The tin–Hb coordination product shows higher affinity towards the 4th gaseous ligand The protons delivered by hydrolysis of tin tetrachloride activate the iron-histidine bound and most probably, the electron transfer Tin coordination to the protein also contributes to the electron transfer process, accelerating its rate

A complete elucidation of the complex interaction between tin tetrachloride and human Hb forms needs further investigations regarding the protein structure after tin coordination, the state of the proximal histidine and of the iron-histidine bond as well as concerning the electron transfer pathways

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