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Mechanochemical reactions of fluorides with hemin

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Abstract

Hemin has two potential sites to react with fluorides, the peripheral acid groups and the central Fe^{III} cation. The mechanochemical reactions of hemin with fluorides (LiF, NaF, KF, CsF, NH_4F and AgF) were monitored using X-ray diffraction (XRD), and IR and Mössbauer spectroscopies. LiF and NaF were found inert when milled with hemin, however KF, CsF, NH_4F and AgF react at both hemin sites. At the iron site Cl^- is replaced by F^- with formation of KCl, CsCl, NH_4Cl, and AgCl, as detected by XRD, while with the peripheral acid groups, fluorides collect the acidic protons to form KHF_2, $CsHF_2$, NH_4HF and $AgHF_2$. The reactions of hemin with the reactive fluorides take place first at the iron site and then at the propionic acid groups. © 2002 Elsevier Science B.V. All rights reserved.

Keywords: Mechanochemical reactions; Hemin with fluorides; Hemoglobin

1. Introduction

Hemoglobin, the oxygen carrier of blood, is composed of a large protein moiety, globin, linked to a Fe^{III}-porphyrin IX center, the heme, by the coordination of Fe to a histidine residue. The hemin molecule, depicted in Fig. 1a, has been used as a simple model to understand hemoglobin chemistry and as a starting reagent in the synthesis of pharmaceutical products for the treatment of anemia [1,2]. It has the Fe^{III} atom coordinated to the porphyrin IX ring. According to the crystallographic studies [3] the Fe^{III} atom is slightly out the plane formed by the porphyrin ring (Fig. 1b). The fifth ligand in axial position is loosely coordinated to a Cl^- anion, while the sixth axial position is vacant for coordination to oxygen and other nucleophylic ligands.

Hemin has two potential sites to participate in reactions with basic substances, the two peripheral acid groups and the central Fe^{III} cation. These reactions are important in the study of hemoglobin biochemistry as well as in the synthesis of complexes with pharmaceutical properties in the treatment of anemia [4,5]. The two propionic acid residues can interact by hydrogen bonding with other OH groups and Fe atoms in neighbor molecules leading to dimeric and polymeric structures [6]. The modification of these interactions is paramount in the synthesis of pharmaceuticals capable to be transported by the blood and assimilated by the cells [7]. The studies of bonding properties of iron at the sixth axial position with a wide variety of ligands have been related with its role as oxygen carrier in biological systems [8]. The absolute majority of reactions with hemin have been carried out in solutions with only a minimum in the solid state [8]. However, the reactions in solid state can shed light on some particularities of hemin complexes of biological importance [8].

In this communication we report reactions of hemin with fluorides by milling together the solid reagents. As reference, the reactions were also carried out in methanolic solutions. The reactions products were characterized by IR, XRD and Mössbauer techniques.

2. Experimental

Li, NaF, KF, CsF, NH_4F, AgF and hemin were commercial reagents of analytical grade (from Sigma) and methanol absolute (from Merck). For IR measurement KBr of spectroscopic grade (also from Sigma) was used. As reference compounds, $LiFHF_2$, $NaHF_2$ and KHF_2 were prepared from HCl and the corresponding fluorides and then purified from methanolic solutions. Their nature and purity were established using IR spectroscopy and XRD.

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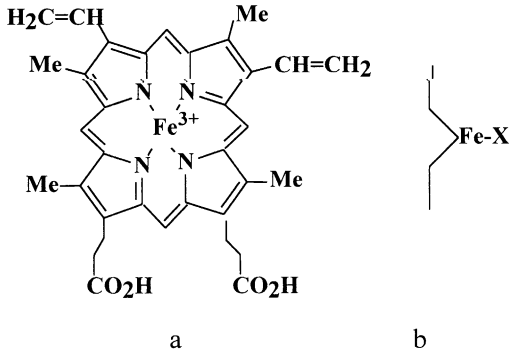

Fig. 1. Structure of (a) Fe^{III} protoporphyrin IX, (b) position of Fe relative to the plane of the porphyrin ring ($X = Cl^-$).

Solid mixtures of hemin to fluorides in 1:1, 1:3, 1:4 and 1:5 molar ratios were prepared and milled in an agate mortar for times from 5 to 20 min and then studied by IR and XRD techniques. Representative milled samples were also studied by Mössbauer spectroscopy. When was needed, the milled mixtures were kept in a desiccator to remove hydration water. A parallel study was also carried out dissolving the reagents in methanol and then evaporating the methanol to dryness. These molar ratios of the reagents were selected in order to defect the preference of the reaction for one or another reaction site of hemin.

IR spectra were recorded in a Bruker FT-IR spectrophometer model Equinox 55 using the KBr pressed disk techniques, except for the reaction with AgF where IR were also recorded in Nujol mulls. KBr can react with silver compounds changing the nature of the studied sample [9,10]. XRD powder patterns were collected with monochromated CuKα radiation using a D8 Advance diffractometer (from Bruker). The indexing of the XRD pattern assigned to $AgHF_2$ was carried out with the TREOR 90 program [11] and the refinement of the lattice parameters with the LSUCRI program [12].

The Mössbauer spectra were obtained at room temperature with a constant acceleration spectrometer in the transmission mode and a ^{57}Co in Rh source. All spectra were fitted using an iterative least-squares minimization algorithm and pseudo-Lorentzian line shapes to obtain the values of isomer shifts (δ), quadrupole splitting (Δ), linewidth (Γ) and the relative line area (A). The isomer shift values are reported relative to sodium nitroprusside at room temperature.

3. Results and discussion

As was stated above, the hemin can react with the fluorides at the central Fe^{III} atom and at the propionic acid residues in the periphery of the porphyrin ring. We will discuss the results according to these two possible reaction sites.

3.1. Reaction at the peripheral acid groups

Alkaline fluorides can react with acidic substances even in solid state to form the complex anion $(FHF)^-$ and the corresponding salt of the acid substance [13,14]. $LiHF_2$, $NaHF_2$, NH_4HF and KHF_2 form crystalline compounds and their XRD powder patterns are known [15]. The reaction with the propionic acid groups of hemin could be sensed by the XRD powder pattern of the milled mixture, for the case of LiF, NaF, NH_4F and KF but also by its IR spectrum for any of the studied fluorides. If a reaction with fluoride takes place the ν(C=O) vibration of the carboxylic acid groups of hemin, which falls at 1704 cm^{-1}, disappears and bands of carboxylate appear at 1563 and 1543 cm^{-1} (a doublet assigned to $\nu_{as}(CO_2^-)$) and single band at 1399 cm^{-1} due to $\nu_s(CO_2^-)$ [8]. The complex anion $(FHF)^-$ also has characteristic absorption bands in the IR spectrum due to its two IR active vibration modes (ν_2 and ν_3) [16], which in our reference sample of KHF_2 were observed at 1255 and 1523 cm^{-1}, respectively (see Fig. 2c). We have noticed in our samples a weak band at 743 cm^{-1} which could be due to the IR inactive ν_1 which becomes allowed by deformation of the linear $(FHF)^-$ in solid state. IR spectra of acidic fluorides also show an absorption band at 2100 cm^{-1}, which we have attributed to a strong hydrogen bonding interaction of the

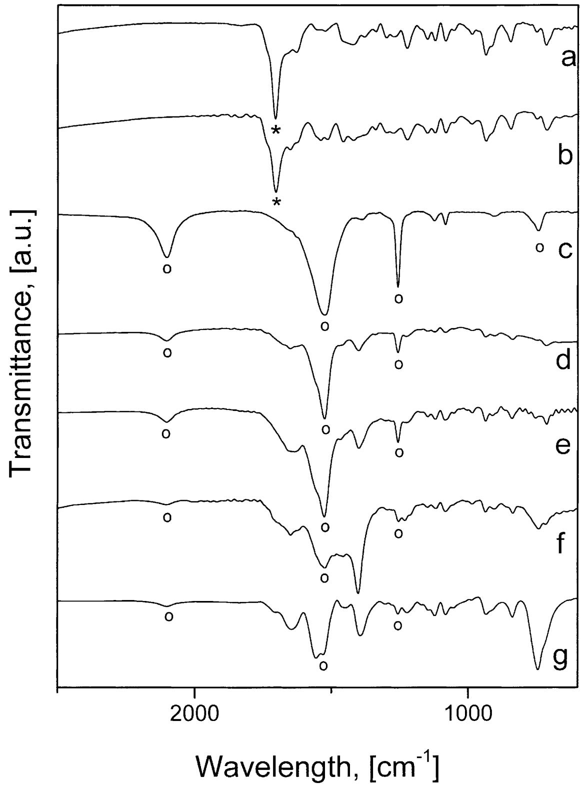

Fig. 2. IR spectra (2300–600 cm^{-1}) of (a) hemin, (b) hemin + NaF, (c) KHF_2, (d) hemin + KF, (e) hemin + CsF, (f) hemin + NH_4F, (g) hemin + AgF. The spectra of the milled samples correspond to mixtures of 1:5 molar ratio of hemin to fluoride. Indicated are the carbonyl vibration of hemin (∗) and the main absorption bands of the acid fluorides (○).

$(FHF)^-$ anion with hydration water, always present in acidic fluorides (see Fig. 2). This band disappears when the sample becomes anhydrous by heating (above 350 °C for $NaHF_2$, for instance). This band at $2100\ cm^{-1}$ is an appropriate sensor to detect acidic fluorides since this region of the IR spectra is free of absorption from groups present in hemin or in the starting fluorides.

In the milled mixtures of hemin with LiF and NaF no changes were observed in the IR band of hemin used as sensor, neither bands of $(FHF)^-$ appear (see Fig. 2). No formation of $LiHF_2$ or $NaHF_2$ was detected in the corresponding XRD powder patterns. In LiF the ionic pair forms a strong bonding which explains its high insolubility and also its inert character in mechanochemical reactions even with the very strong oxalic acid [13]. It seems that the carboxylic groups of hemin are not as acid to participate in a proton transfer reaction with NaF. When NaF is milled with oxalic acid the acidic sodium oxalate is formed indicating that NaF only can remove very acidic protons [14]. When methanolic solutions of hemin and NaF were mixed and the methanol allowed to evaporate, in the final product only the starting reagents were detected indicating that the presence of the solvent does not favor the reaction.

KF, CsF, NH_4F and AgF showed a different behavior than NaF and LiF when reacted with hemin. In the IR spectra of the 1:4 molar milled mixtures always formation of carboxylate and $(FHF)^-$ are observed (see Fig. 2). These IR spectra show the band at $2100\ cm^{-1}$, which is characteristic of acidic fluorides. In accordance with the IR results, XRD powder pattern of the sample milled with KF (see Fig. 3) show formation of KHF_2 (PDF 48-1667) [15]. With AgF, in addition to the XRD powder pattern of AgCl due to the reaction at the iron site (discussed below), a set of diffraction peaks appears which, accordance to the IR evidence, were attributed to formation of $AgHF_2$ (see Fig. 3). This set of diffraction peaks was indexed (see Table 1) resulting in a tetragonal cell with cell edges, $a = b = 8.178$ Å, $c = 4.966$ Å and a figure of merit $M(9) = 28$. KHF_2 also crystallizes in a tetragonal cell with cell edges $a = b = 5.686$ Å and $c = 6.829$ Å (PDF 48-1667) [15]. XRD patterns of the milled mixtures with NH_4F and CsF (in a 1:5 molar ratio) only show very weak diffraction peaks that could be assigned to NH_4HF_2 (PDF 12-0302) and $CsHF_2$, respectively. It seems that in the studied samples these two acidic fluorides show a poor crystallinity to be detected by XRD within the obtained hygroscopic paste.

According to the above discussed evidence, the reactions at the peripheral acidic groups proceed as follow:

$$R{-}(CO_2H)_2 + 4KF \rightarrow R{-}(CO_2K)_2 + 2KHF_2 \quad (1)$$

$$R{-}(CO_2H)2 + 4CsF \rightarrow R{-}(CO_2Cs)_2 + 2CsHF_2 \quad (2)$$

$$R{-}(CO_2H)_2 + 4NH_4F \rightarrow R{-}(CO_2NH_4)_2 + 2NH_4HF_2 \quad (3)$$

$$R{-}(CO_2H)_2 + 4AgF \rightarrow R{-}(CO_2Ag)_2 + 2AgHF_2 \quad (4)$$

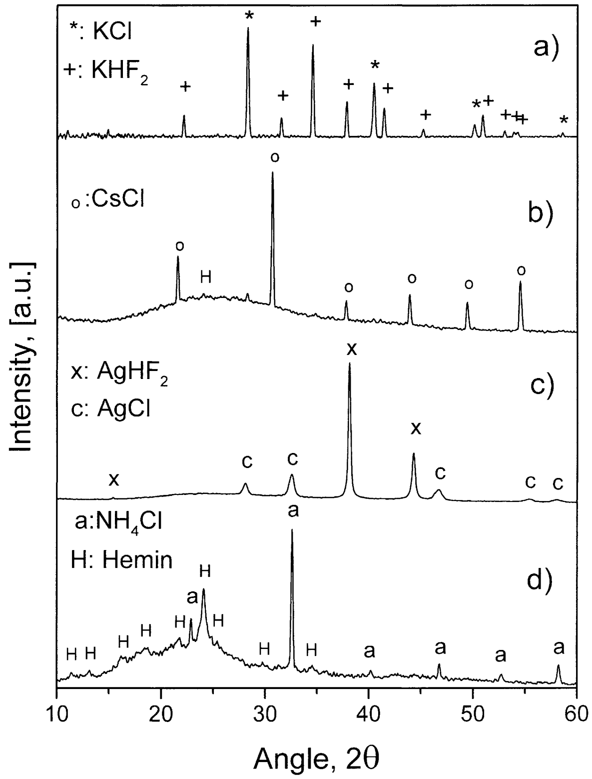

Fig. 3. XRD powder patterns of the milled samples: (a) hemin + KF (1:5); (b) hemin + CsF (1:5); (c) hemin + AgF (1:5); (d) hemin + NH_4F (1:5). Indicated is the molar ratio of the mixtures.

3.2. Reaction at the central iron site

The reactions at the iron site were monitored by the XRD powder pattern of the milled mixtures. Even when the reactions of hemin with fluorides here studied are mainly carried out in solid state, in the following we will use the concept of insolubility as a measure of stability of a given compound and also of the forces that held together their atoms. With the exception of Li^+ and Na^+, chlorides of the considered alkali cations are more insoluble than the corresponding fluorides [17]. In consequence, an exchange of Cl^- for F^- is expected at the Fe^{III} site when hemin is milled with

Table 1
X-ray powder diffraction data of $AgHF_2$[a]

h	*k*	*l*	*d* (Å)$_{Obs}$	*d* (Å)$_{Cal.}$	I/I_{100}
1	1	0	5.781	5.7827	1
1	0	2	2.376	2.3761	100
4	0	0	2.044	2.0445	34
5	2	1	1.452	1.4522	23
5	4	1	1.237	1.2369	21
2	0	4	1.188	1.1880	6
4	2	4	1.027	1.0272	1
8	1	2	0.939	0.9390	6
6	0	4	0.918	0.9178	6

[a] Unit cell: tetragonal; cell parameters: $a = b = 8.178(3)$ Å, $c = 4.966(3)$ Å; figure of merit $M(9) = 28$.

KF, CsF, NH_4F and AgF. XRD powder patterns of the milled mixtures agree with these expected results. With LiF and NaF only the patterns of these salts and of partially amorphous hemin were observed. With KF, CsF, NH_4F and AgF in the 1:5 molar milled mixtures always KCl (PDF 41-1476), CsCl (PDF 05-0607), NH_4Cl (PDF 73-0365) and AgCl (PDF 31-1238), are detected (see Fig. 3). CsCl is detected even in the humid milled mixture (Fig. 3b). Mössbauer spectrum also senses the replacement of Cl^- for F^- in the coordination sphere of the Fe^{III} atom. The isomer shift (δ) and the quadrupole splitting (Δ) of the unresolved doublet change from 0.59 and 0.86 mm/s in hemin to 0.61 and 0.63 in hemin milled with KF, respectively (errors in these values are no higher than ± 0.02 mm/s). These results agree with the reported Mössbauer data for hemin with different anions instead Cl^- [18,19]. F^- has a stronger σ interaction with Fe^{III} than Cl^-, which is compensated by a higher π-back bonding electron subtraction from Fe^{III} toward porphyrin nitrogens. This last effect favors the bonding of iron to the porphyrin ring and reduces the axial elongation which is observed as a smaller Δ value for F^--hemin when compared with that of Cl^--hemin.

3.3. Preference of fluorides for one of the reaction sites of hemin

Because in hemin there are two reaction sites, the reaction must proceed preferentially in one site and then in the other one. With AgF the reaction first takes place at the iron site due to the high insolubility (stability) of AgCl. For the 1:1 molar mixture containing AgF the XRD shows the pattern of AgCl (see Fig. 4). For KF, CsF and NH_4F there are two competitive driving forces, the higher stability of KCl, CsCl and NH_4Cl versus KF, CsF and NH_4F, respectively; and the ability of fluorides to collect protons from the acid groups to form the anion $(FHF)^-$. In addition, hemin has two acid groups, and probably hemin behaves as any other diprotic acid, which have two different ionization potentials [20]. When hemin was milled with KF in a 1:1 molar ratio the XRD pattern indicates formation of KCl and not of KHF_2 (see Fig. 4) while for the 1:2 mixture both, KCl and KHF_2 are detected. The reaction with KF first takes place at the iron site and then with the propionic acid groups. A similar result is obtained with CsF and NH_4F (see Fig. 4).

In a reaction of hemin to fluoride with a 1:3 ratio one fluoride molecule must react at the iron site while the remaining two molecules must participate in a proton transfer reaction with one of the acid group of hemin. Since in hemin the acid groups of two neighbor hemin molecules must be linked by hydrogen bonding interactions, the reaction at one of these acid sites would disrupt that interaction. In that case the ν(C=O) vibration could be observed above 1720 cm^{-1} due to the monomeric CO_2H group. However, our experiments fail to demonstrate this hypothesis. In the 1:3 milled mixture the ν(C=O) vibration was observed at 1704 cm^{-1}, as in the starting hemin. Therefore, only one acid group per hemin is neutralized and the other one remains linked to other hemin molecule by hydrogen bonding interactions.

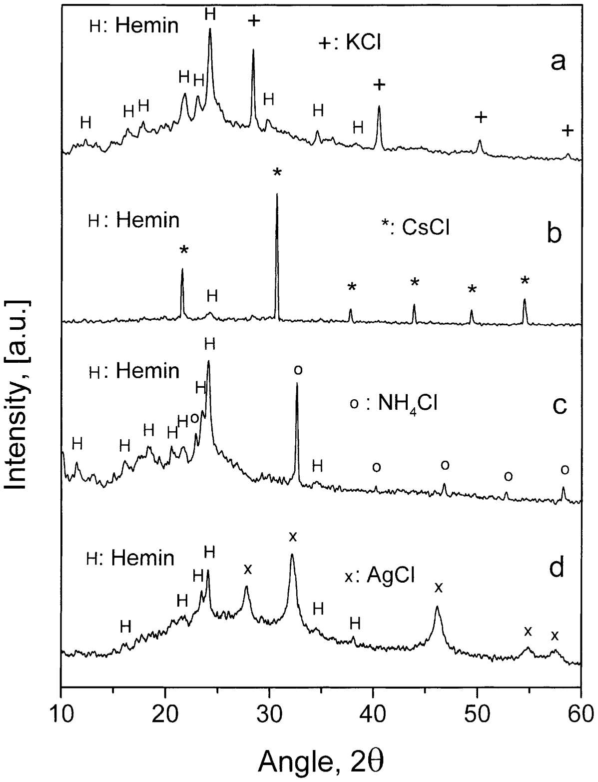

Fig. 4. XRD powder patterns of the milled samples: (a) hemin + KF (1:1); (b) hemin + CsF (1:1); (c) hemin + NH_4F (1:1); (d) hemin + AgF (1:1). Indicated is the molar ratio of the mixtures.

No significant differences were found in the end products of the reactions carried out in solid state (by milling) and of those from methanolic solutions. However, due to the hygroscopic character of fluorides (except LiF and NaF) and the corresponding acid fluorides, the reactions in solid state are preferable to those in solutions.

4. Conclusions

Mechanochemical reactions of hemin with fluorides (KF, CsF, NH_4F and AgF) produce the neutralization of the acid groups of hemin leading to the corresponding salt, with the formation of the proper acid fluoride (KHF2, $CsHF_2$, NH_4F and $AgHF_2$), while at the iron site the Cl^- anion of hemin is replaced by F^- with the formation of the corresponding chloride (KCl, CsCl, NH_4Cl and AgCl). These reactions first take place at the iron site and then at the peripheral acid groups. LiF and NaF were found inert when milled with hemin.

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