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Induced fit activity-based sensing: a mechanistic study of pyrophosphate detection with a "flexible" Fe-salen complex†

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Activity-based sensing of biological targets is attracting increasing attention. In this work, we report detailed UV-Vis and fluorescence mechanistic studies on an Fe-salen based probe, [Fe III(salenMeCl2(SO 3) 2)OH] 3− for pyrophosphate (PPI) detection. In the presence of PPI as an analyte, the probe disassembles into its molecular subunits and releases a fluorescent signal. Our studies illustrate that the aqua form of the complex (1-OH) 2 is the active species and that upon substitution of Fe-coordinated H 2 O and an initial end-on coordination of HP 2 O 7 2− , the “trapped” pyrophosphate species switches from a monodentate to a bidentate coordination mode (i.e. linkage isomerism) via a probable equilibrium process. The elusive intermediate is further stabilized by a hydrogen bonding interaction that activates the probe for the subsequent final irreversible rate-limiting step, and allows selective discrimination between the other pyrophosphate (H 2 P 2 O 7 2− and P 2 O 7 4−) species in favour of the HP 2 O 7 2− . The flexible mode of molecular recognition and binding of HP 2 O 7 2− by the tetradentate probe 1-OH 2 is unexpected and most effective at physiological pH, and has precedence in enzymatic catalysis (i.e. induced fit principle). These binding properties explain the previously observed outstanding selectivity of 1-OH 2 for pyrophosphate over other (poly)oxophosphates and potentially competing analytes.

Introduction

Activity-based sensing (ABS) attracts increasing attention for elucidating the roles and importance of chemical species in biological systems. 1,2

In this context, enormous progress has been observed in the development and application of chemosensors for detecting small reactive molecules of the carbon-, 3,4 oxygen-, 5,6 nitrogen-, 7,8 and sulfur families. 6,8,9 In contrast to these developments, chemical methods for sensing phosphorus-containing compounds in biological environments are scarce. 10-13 Phosphates are ubiquitous in biological systems and play pivotal structural and functional roles ranging from its occurrence in the skeleton to signal transduction and energy storage processes. 1,4,15 These biologically important anions are present in multiple facets and phosphates are encountered in different protonated forms of inorganic mono- and polyanions or as covalently bound building blocks of natural products such as pyrophosphate (PPI), 16 adenosine triphosphate (ATP) or vitamin B 12. 17,18 For example, H 2 P 2 O 7 is a four-proton donor with pK a values of 0.85, 1.96, 6.60 and 9.41 19 and hence, at physiological pH of 7.40, PPI is encountered as a mixture of HP 2 O 7 3− (86%) and H 2 P 2 O 7 4− (14%). 11,20

The Zelder group and others are interested in the development of activity-based probes to detect and study the roles of phosphates in biological systems, in the environment and in foodstuffs. 11,21-23 For this purpose, fluorescent probes are advantageous, but selective sensing of the target analyte in the presence of a pool of related bioavailable, competing phosphates is highly challenging. 24-27 Genetically encoded and aptamer based systems offer interesting opportunities, 28-30 whereas cost-effective small-molecular chemical systems are still rare. Important examples of the latter include a boronic acid functionalized rhodamine derivative for detecting mitochondrial ATP as well as Zn-terpyridine system for sensing cellular PPI. 27,31 Two of us introduced Fe-salen complexes for selectively detecting PPI in cancer cells and in foodstuffs. 11,21,23 The Fe III complex 1-OH 2 (Fig. 1A) exhibits a square pyramidal coordination geometry and is composed of four (i-iv) subunits: 23 a central Fe III ion (i), a 1,2-propanediamine backbone (ii), two 3-chloro-5-sulfoisalicylaldehyde moieties (iv) and (iii)
and an apical water ligand (iv; Fig. 1A). Upon interaction with PPi, the metal complex disassembles and unleashes the fluorescent signaling unit 4 (Fig. 1A). Importantly, discrimination between PPi and other anions including related phosphates such as inorganic phosphate, ATP and ADP was demonstrated. Mechanistic details on the nature of the active species, the molecular recognition and the binding event as well as the irreversible disassembly process have however not been studied so far.

Thus, herein we present a detailed kinetic-mechanistic study on the structurally “flexible” probe 1-OH₂ for the fluorometric detection of pyrophosphate (Fig. 1). In particular, we
Experimental section

Materials
Complex 1-OH$_2$ was synthesized as its sodium salt as reported earlier. Sodium salts of PPi and F$^-$ were used in the kinetic studies. Unless otherwise stated, all chemicals were of reagent grade and purchased from Sigma-Aldrich-Merck, Fluka, Apollo Scientific or Alfa Aesar. Solvents for reactions were of p.a. grade. Stock solutions for kinetic experiments were prepared in Milli-Q H$_2$O.

Instrumentation
pH: Metrohm 827 pH meter equipped with pH sensitive electrode was employed. UV-Vis spectra: Cary Series Spectrophotometer (Agilent technologies); $\lambda_{\text{max}}$ in nm. UV spectra were recorded between 230 and 800 nm at 1.2 nm resolution and 20 points per s. Fluorescence spectra: Luminescence spectrometer, PerkinElmer, LS 50B. Unless stated, the slit-widths for the fluorescence experiments were kept at 10 nm (excitation) and 10 nm (emission) and the excitation wavelength was set at 385 nm. Unless stated, a UV Fused Silica Metallic Neutral Density Filter (FSQ-ND05) with optical density of 0.5 (from Newport) was used for fluorescence studies.

Kinetic studies and data treatment
All UV-Vis and fluorescence kinetic experiments were carried out in a quartz cuvette (3.5 mL, 10 mm) without protection from air under pseudo first-order reaction conditions. Unless otherwise stated, all kinetic experiments were carried out with complex 1-OH$_2$ (16 mM) at pH 7.35 ([Tris buffer] = 10 mM) at five different temperatures (12, 18, 25, 32 and 38 °C) and in presence of seven different concentrations of PPi (0.16, 0.24, 0.32, 0.48, 0.56, 0.64 and 0.80 mM). pH studies were performed at thirteen different pH values: 4.50, 5.00, 5.36, 5.71, 6.02, 6.33, 6.70, 7.00, 7.35, 7.67, 8.12, 8.42 and 9.40 ([Britton Robinson buffer] = 10 mM). Absorption and emission traces were collected over time until completion of reaction indicated by saturation. Origin 2020 and Micromath Scientist$^{24}$ were used for fitting the kinetic traces and other specific functions. The observed pseudo first-order rate constants were obtained from least-squares (L.S.) fits of absorbance at 385 nm and emission at 500 nm ($\lambda_{\text{ex}} = 385$ nm) vs. time traces to an appropriate first-order exponential, as described previously and illustrated in Fig. 3 and ESL. In Fig. 3–6, the solid lines indicate the L.S. fits/simulations of the respective experimental data (shown as individual points) to appropriate functions (details shown in the ESI$^\dagger$).

Results and discussion

PPI-triggered disassembly of complex 1-OH$_2$

The PPI-triggered disassembly of 1-OH$_2$ into the signaling unit 3-chloro-5-sulfosalicylaldehyde (4), 1,2-propanediamine (6) and Fe(PPi)$_4$ (5) (Scheme 1) was established previously based on the following observations. Firstly, upon addition of PPi (10 equiv.; 0.16 mM) to an aq. soln. of 1-OH$_2$ (16 mM), the formation of a new absorption band at 385 nm was observed in the UV-Vis spectrum and a simultaneous 5-fold enhanced emission at 500 nm was observed with fluorescence spectroscopy (see also Fig. 3). These characteristic spectroscopic changes were unambiguously attributed to the liberation of 4 from complex 1-OH$_2$. Moreover, these observations were supported by mass spectrometric and $^1$H NMR studies. In the latter experiments, formation of 4 from 1-OH$_2$ (2 mM) was observed within 30 min at pH 7.35 ([Tris buffer] = 10 mM) after the addition of PPI (10 equiv.; 20 mM). Remarkably, we did not obtain any evidence for the intermediate formation of the metal-free salen ligand from disassembly experiments in deuterated H$_2$O (Fig. 2).

Kinetic studies of the disassembly mechanism

Fig. 3A reports UV-Vis spectral changes for the reaction between complex 1-OH$_2$ (16 mM) and PPI (30 equiv.; 0.48 mM) at pH 7.35 ([Tris buffer] = 10 mM) and 25 °C as a function of time. Changes of absorbance at 385 nm indicate the release of the signaling unit 4 and depict a first-order behavior with a rate constant of $k_{\text{obs(ABU)}} = (1.025 \pm 0.008) \times 10^{-5}$ s$^{-1}$ and a half-life of ca. 600 s (approx. 10 min) (Fig. 3 inset). Also shown in Fig. 3 is the concurrent fluorescence changes at 500 nm ($\lambda_{\text{ex}} = 385$ nm) with time (at 28 °C), which is clearly very similar to that observed from the absorbance measurements with $k_{\text{obs(Fluo)}} = (1.30 \pm 0.03) \times 10^{-3}$ (single exponential) and $k_{\text{obs(Fluo)}} = (1.280 \pm 0.006) \times 10^{-3}$ (two exponential) s$^{-1}$, respectively, taking into account the slight temperature increase.
Five isosbestic points were observed in the Abs vs. time spectra at 253, 265, 287, 350 and 420 nm, (Fig. 3), potentially indicating a clean conversion to the final product(s).

The absorbance vs. time traces under the selected experimental conditions showed clear first-order changes, although preceded by earlier reactions, which exhibit corresponding small absorbance/fluorescence changes. Following the principal reaction, an additional process (more prominent at higher pH) is depicted as a two-exponential function (Fig. 4; red line) although a single exponential function is expected to show at least a first-order dependence on the PPI as entering nucleophilic species. Finally, the overall reaction is expected to be a multi-step process, as depicted in Scheme 1 (vide infra).

Subsequently, the influence of pH on the reaction rate with fixed concentration of [PPI]tot and [1]tot was studied at 25 °C between pH 4.50 and 9.40 and the results are illustrated in Fig. 4 and the ESI (Fig. S1, Table S1). The pseudo first-order rate constants (kobs) as a function of pH depict a quite well-defined bell-shaped curve with a maximum around pH 7.00 (Fig. 4 blue line and blue data points) resembling a similar shape as the speciation curve of HP2O3– with a pKα of 6.60.21 (Fig. 4; red line). However, the pH at which the maximum value of the pseudo first-order rate constant is reached deviates from the pH value at which the maximum distribution of the HP2O3– is found (i.e. ~pH 8.00). This behavior is nicely explained by considering only the aqua anionic hydroxido species, 1-OH, expected to show at least a first-order dependence on the PPI while 1-OH2 has one pKα value, forming the doubly charged anionic hydroxido species, 1-OH–. Moreover, it is further a logic conclusion that 1-OH2, upon reacting with the PPI, is expected to show at least a first-order dependence on the PPI as entering nucleophilic species. Finally, the overall reaction is expected to be a multi-step process, as depicted in Scheme 1 (vide infra).

Fig. 2 Line 1: 1H NMR spectra of 1-OH2* (2 mM) in the presence of PPI (10 equiv.; 20 mM; incubation time: 30 min) at pH 7.35 (D2O, Tris buffer = 10 mM). Line 2: 1H NMR spectra of pure signaling aldehyde 4 in D2O. Characteristic protons are indicated in Fig. 1A. * The 1H NMR spectrum of 1-OH2 did not show any signal in this region due to the paramagnetic nature of the probe.

Fig. 4 and the ESI (Fig. S1, Table S1) show the reaction scheme for the disassembly as studied kinetically, with reactant species 1-OH2 and 1-OH (interrelated by the acid dissociation constant Kα and with [1]tot). Compounds [2] and [3] are assumed reactive intermediates, 5 = final Fe(PPi)6 species and 4 = 3-chloro-5-sulfosalicylaldehyde. [PPI]tot = total pyrophosphate concentration, of which the parent tetra-acid exhibits four pKα values, denoted by Kα1, Kα2, Kα3 and Kα4. The exact pH and entering nucleophile dependencies of these reactions with the required accuracy, these could nevertheless be interpreted as confirmation of the preceding equilibria K1 and K2 as indicated in Scheme 1.

It is expected that the rate law for the overall process should display pH dependent behavior since, as indicated above, the pyrophosphoric acid exhibits four successive pKα values,19 while 1-OH2 has one pKα value, forming the doubly charged anionic hydroxido species, 1-OH–. Consequently, it is further a logic conclusion that 1-OH2, upon reacting with the PPI, is expected to show at least a first-order dependence on the PPI as entering nucleophilic species. Finally, the overall reaction is expected to be a multi-step process, as depicted in Scheme 1 (vide infra).

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Scheme 1 Simplified reaction scheme for the disassembly as studied kinetically, with reactant species 1-OH2 and 1-OH (interrelated by the acid dissociation constant Kα and with [1]tot). Compounds [2] and [3] are assumed reactive intermediates, 5 = final Fe(PPi)6 species and 4 = 3-chloro-5-sulfosalicylaldehyde. [PPI]tot = total pyrophosphate concentration, of which the parent tetra-acid exhibits four pKα values, denoted by Kα1, Kα2, Kα3 and Kα4.
nucleophilic for substituting the aqua ligand in complex 1-OH$_2$ ($pK_a = 6.90$) as indicated by the rapid decrease of the reaction rates at more acidic pH values ($<pH 6.00$). This underlines the novel ability of 1-OH$_2$ to selectively discriminate between the H$_2$P$_2$O$_7^{2-}$/P$_2$O$_7^{4-}$ anions (both present in significant amounts in the pH range studied) in favor of HP$_2$O$_5^{3-}$. Moreover, it agrees with the fact that at more basic pH values, even the presence of the highly charged P$_2$O$_7^{4-}$ species ($pK_a = 9.41$) does not trigger the disassembly reaction of 1-OH$_2$, which might in turn suggest that hydrogen bonding in the ring closing step is important (see further below). It should be noted that there is an indication of a slow parallel reaction (data points at ca. pH 8.00–9.00), although it clearly does not significantly affect neither the specificity nor the selectivity of 1-OH$_2$.

Having determined the aqua form of Fe-complex (1-OH$_2$) and HP$_2$O$_5^{3-}$ as the active species in the disassembly reaction, we studied the influence of increasing concentration of [PPI]$_{tot}$ on the reaction rate at the pH where the capturing of the HP$_2$O$_5^{3-}$ is the most effective. Variations of [PPI]$_{tot}$ at pH 7.35 and 25 °C (Fig. 5 blue graph) provide evidence of faster, preceding reactions, $i.e.$, saturation kinetics with a rate-limiting dependence on [PPI]$_{tot}$. The saturation kinetics provides excellent support for concluding preceding reactions, as postulated in Fig. 1 and Scheme 1. This behavior is thus supporting a rapid overall pre-equilibrium reaction, denoted by $K_{eq}$ and within this report indicated to be the net of initial coordination ($K_a$) followed by a linkage isomerism ($K_s$) succeeded by a final rate limiting first-order formation of products 4 and 5 (Scheme 1), denoted by $k_{PPI}$. Based on these observations and the detailed study of the following systematic kinetic experiments, we summarize the mechanism of the PPI-triggered disassembly of Fe-salen complex 1-OH$_2$ into its molecular building blocks 4, 5 and 6 in Scheme 1.

The total rate law may be derived from Scheme 1, yielding the complete expression of the pseudo first-order rate constant in eqn (1), which describes all the observations made in this study related to the total process.

$$k_{obs} = k_{PPI}K_{eq}[HP_2O_5^{3-}]/[I + (K_a/[H^+])] + (K_{eq}[HP_2O_5^{3-}])$$  \hspace{1cm} (1)$$

In eqn (1), the concentration of the tri-anionic species HP$_2$O$_5^{3-}$ is given by eqn (2).

$$[HP_2O_5^{3-}] = ([PPI]_{tot}(K_a/[H^+])}/[I + (K_a/[H^+])] + (K_{eq}[K_{a4}/[H^+]])$$  \hspace{1cm} (2)$$

In particular, the net total reactivity and specificity of 1-OH$_2$ towards the tri-anionic HP$_2$O$_5^{3-}$ species is well addressed in eqn (1), when [PPI] $\gg$ [1]$_{tot}$, and clearly describes
(a) The total acid–base behavior of both the PPI and the 1-OH\textsubscript{2} species (Fig. 4);
(b) The plateau reached at high [PPI\textsubscript{tot}] (Fig. 5);
(c) The simultaneous net changes in absorbance and fluorescence induced (Fig. 3).

(d) The small, potential concurrent reaction (\(k_{22}\), Fig. 4; caption) to the final step gives a value of zero within standard deviation, not justifying inclusion, see Fig. 5 and 6.
Finally, it should be noted that in eqn (1) and (2), only $K_{a1}$, $K_{a2}$ and the acid dissociation constant of the 1-OH$_2$ species ($K_a$) are included, since at the pH values evaluated ($\text{pH} > 4$) the total PPI equals the concentration of the di-anionic species, H$_2$P$_2$O$_7^{2-}$, which then dissociates further as the pH increases. Additional and detailed information relevant to the complete derivation of eqn (1) is given in the ESI (sections S1.1 and S2).

Based on Scheme 1, and on the observation that virtually identical pseudo first-order rate constants are obtained from the absorbance/fluorescence vs. time data (Fig. 3), we proceeded to study the effect of a variation of [PPI] on both these properties in more detail. This is illustrated in Fig. 5 and confirms that similar individual rate constants are obtained, and that the same $k_{\text{obs}}$ vs. [PPI] profile is observed from both the absorbance/fluorescence vs. time data, yielding limiting kinetics as predicted by eqn (1).

The disassembly reactions of 1-OH$_2$ and PPI were performed at six different temperatures and different concentrations of [PPI]$_{\text{tot}}$ (Fig. 5; Fig. S2–S4, Table S2†). As expected, the individual pseudo first-order rate constants increase with increasing temperatures (Fig. 5) and a least-squares (L. S.) fit analysis resulted in the determination of the equilibrium constant $K_{\text{eq}}$ of (4300 ± 400) M$^{-1}$ and the first-order rate constant at 25 °C $k_{\text{eq}}$ of (3.2 ± 0.2) $\times$ 10$^{-3}$ s$^{-1}$ (Table 1). This agrees very well with that obtained from the global fit (Table 1; Fig. S6†), as well as from the pH fit reported in Fig. 4. The latter defines the rate-determining step in reaction (IV) (Scheme 1), that is the formation of the signaling unit 4, and 5. We assume that the latter is a Fe-pyrophosphate species in agreement with previous investigations (Scheme 1).32 Results of the rate determining reaction (IV) were obtained by extended UV-Vis studies and are summarized in Fig. 5 and Table 1.

In an attempt to investigate the rapid pre-equilibrium ($K_{\text{eq}}$) of the reaction between 1-OH$_2$ and [PPI] in more detail, we analyzed deviations at the isosbestic point at 287 nm in the UV-Vis spectra in more detail (Fig. 6). We selected the lowest reaction temperature (12 °C) in an attempt to slow these down as much as possible to ensure accurate analysis thereof.

The subtle changes at this wavelength were observed early in the reaction at 12 °C but could hardly be detected at higher temperatures. The principal changes of absorbance vs. time at this wavelength correlate with reaction (IV) (Scheme 1; Fig. 6), following an “induction period” of <10 min. The latter is assumed to consist of preceding reactions which are attributed to two successive rapid equilibria (reactions (II) and (III); Scheme 2) as postulated in Scheme 1.

Based on this experimental proof, we assign the overall equilibrium constant $K_{\text{eq}}$ to the combination of the two equilibria with individual equilibrium constants $K_1$ and $K_2$ ($K_{\text{eq}} = K_1K_2$) (Schemes 1 and 2). It thus defines the overarching equilibrium from 1-OH$_2$ to [3] via [2], whereafter the disassembly is triggered. We assume that HP$_2$O$_5$$^{3-}$ first substitutes the aqua ligand of 1-OH$_2$ forming [2], in which the pyrophosphate entity acts as a monodentate ligand (Schemes 1 and 2). This binding mode preorganizes the other phosphate subunit of the Fe-bound PPI ligand for chelation via a postulated hydrogen bonding, and hence the PPI switches from a monodentate to a bidentate coordination mode (i.e. linkage isomerism) as depicted in the octahedral complex [3] (Schemes 1 and 2).30,53–55 Both intermediates have been confirmed by DFT calculations and intermediate [3] was found to be stabilized by 34.1 kcal mol$^{-1}$ compared to the initial monocoordinated intermediate [2] (Fig. S7 and S8, section S5†). This is in agreement with the equilibrium also lying towards [3] compared to [2]. Such bidentate coordination mode has been recently observed in a Fe$^{III}$phenanthonol-amino complex.56 Some precedence for strong hydrogen bonding at the O-donor atoms in tetradeutated ligands is also found in the commercial $^{99m}$Tc-radiopharmaceutical imaging agent, Ceretec, utilizing the multidentate hexamethylpropylene amine oxime ligand.57,58

It is therefore postulated that intermediate [3] is activated for the irreversible disassembly into its molecular building blocks by a hydrogen bond between the $\kappa^2$-O,O’ pyrophosphate entity (HP$_2$O$_5$$^{3-}$) and the equatorial phenolato group of the salen ligand (Scheme 2). In fact, hydrogen bonds from hydrogen donors to the phenolate oxygen unit of metal–salen complexes are common structural features.59,60

This hypothesis was supported by accompanying fluorescence kinetic studies as performed and described herein. In particular, we followed the increase of emission intensity at 500 nm during the PPI-triggered disassembly reaction of 1-OH$_2$, and as demonstrated earlier, this signal is univocally attributed to the liberation of the signaling unit 4 (Scheme 1) during the disassembly reaction. In the fluorescence studies, we collected fluorescence scans at 500 nm with time at different concentrations of [PPI]$_{\text{tot}}$ at 28 °C and pH 7.35 (Fig. S5†). The generation of fluorescence correlates exactly with reaction (IV), following the “induction period” of only a few minutes (Fig. 6; see also Fig. 3(C)). The respective $k_{\text{obs}}$ values at different [PPI] are summarized in Table S3† and illustrated in Fig. 5.

In summary it is concluded that although the “induction period” is clearly observed in the very early parts of the reac-

**Table 1** Kinetic data for the disassembly reaction of 1-OH$_2$ with PPI

| Parameter | Temperature (°C) | Value |
|-----------|-----------------|-------|
| $k_{\text{eq}}$ (s$^{-1}$) | 12 | (9.09 ± 0.10) $\times$ 10$^{-4}$ |
| | 18 | (1.66 ± 0.13) $\times$ 10$^{-3}$ |
| | 25 | (3.2 ± 0.2) $\times$ 10$^{-3}$ |
| | 28$^a$ | (3.5 ± 0.7) $\times$ 10$^{-3}$ |
| | 32 | (4.1 ± 0.2) $\times$ 10$^{-3}$ |
| | 38 | (5.5 ± 0.3) $\times$ 10$^{-3}$ |
| | 43 | (9.4 ± 0.5) $\times$ 10$^{-3}$ |
| $K_{\text{eq}}$ (M$^{-1}$)$^a$ | $^d$ | 4300 ± 400 |
| | 25$^c$ | 4300 ± 400 |
| | 28$^a$ | 3500 ± 1100 |
| $\Delta H^\circ$ (kJ mol$^{-1}$) | — | 62 ± 1 |
| $\Delta S^\circ$ (J K$^{-1}$ mol$^{-1}$) | — | −86 ± 4 |

$^a$ Fig. 5. $^b$ Fig. 4; eqn (1), using all $k_{\text{obs}}$ values at 25 °C (both variation of pH and [PPI] used). $^c$ Fluorescence data, see Fig. 5. $^d$ Average from six temperatures: 12, 18, 25, 32, 38 °C (obs vs. time). $^e$ Global fit, see ESI, Fig. S6.$^f$
ation in both the UV-Vis and the fluorescence kinetic studies, the spectral changes were too small and the associated relative standard deviations too large to allow for accurate analysis and was not pursued further.

However, in an attempt to further evaluate the initial substitution of the aqeous ligand of complex $\text{1-OH}_2^-$ (Scheme 2; reaction (II)), we tested a monodentate nucleophile, namely, $\text{F}^-$ (0.16 mM) in kinetic studies at 12 °C and pH 7.35 and in the absence of PPI. These studies resulted only in a slow reaction that we assign to a background hydrolysis of the complex without significant observed coordination of the monodentate ligand to the iron center (Fig. S9†). In addition, utilization of the large excess of $\text{F}^-$ (8.00 mM) in combination with PPI did not lead to substantial changes in the pseudo first-order rate constant of the PPI-triggered disassembly reaction of complex $\text{1-OH}_2^-$ (Fig. S10†), although some changes in the total absorbance changes were indeed observed. This experiment suggests limited competition in the substitution of the aqua axial ligand in $\text{1-OH}_2^-$ by $\text{F}^-$ ions. Nevertheless, since the yield in absorbance was reduced, it indicates that there is some coordination by $\text{F}^-$ nucleophile in a probable parallel process, potentially trapping small amounts of $\text{1-OH}_2^-$ (Fig. S9†). However, since the experiments were performed under pseudo first-order conditions ([PPI] >> $[1]_{tot}$), variations in $[1-\text{OH}_2^-]$ are not expected to influence the total kinetics observed. Moreover, as indicated for the preceding reactions with PPI (Fig. 6), small absorbance changes and large estimated standard deviations in traces with a monodentate nucleophile such as $\text{F}^-$ ions also rendered further accurate evaluation thereof unreliable and was not pursued further.

The initial steps of the disassembly process of the PPI selective probe $\text{1-OH}_2^-$ resemble the “induced fit” binding of a substrate to a protein during enzymatic catalysis, wherein the process is associated with a conformational change of the enzyme–substrate complex. In the disassembly of probe $\text{1-OH}_2^-$, the initial binding of HP$_2$O$_7^{3-}$ is followed by a switch of the monodentate ($\kappa^2$O pyrophosphate) to a “bidentate” coordination mode ($\kappa^2$O$_2$O$' $ pyrophosphate; Schemes 1 and 2). Based on kinetic evidence, we propose that this configurational change of the complex is associated with large conformational variations (also in agreement with the activation parameters, vide infra) that trigger the PPI-induced demetallation and subsequent release of signaling unit 4.

In contrast to observations with $\text{1-OH}_2^-$, most activity-based probes (ABP) follow the “key-lock” principle of molecular recognition for triggering a chemical irreversible reaction after binding of the analyte. We expand this concept herein to “induced fit” ABP that requires a large configurational and/or conformational change upon initial analyte binding (“induced fit” binding) for triggering the disassembly and signal transduction process.

Finally, the activation parameters of $\Delta S^i = -86 \pm 4 \text{ J K}^{-1} \text{ mol}^{-1}$ and $\Delta H^i = 62 \pm 1 \text{ kJ mol}^{-1}$ for the disassembly reaction of $\text{1-OH}_2^-$ induced by PPI were obtained from a global fit analysis (section S1.1 and S6†) of the reactions monitored at different concentrations of [PPI]$_{tot}$ and temperatures as discussed in detail above (Fig. S2-S4 and S6†). The significant negative activation entropy associated with the final, rate-determining step is puzzling since the formation of both 4 and 5 overall might suggest a dissociative process. However, the significant structural changes required for the reorganization of intermediate [2] to [3] for triggering the disassembly (reaction (IV); Scheme 1), which in itself is a multi-step process, is assumed to be the origin of this behavior.

Conclusions

In recent years, the development of activity-based probes for biological analytes has attracted increasing attention. Despite
enormous progress, mechanistic studies on the mode of analyte detection are still rare. Herein, we report a detailed kinetic-mechanistic evaluation on the detection of PPi with an Fe-salen activity-based probe following a disassembly reaction. The data presented herein illustrate that the aqua form of the complex (1-OH₃) is the active species and that upon substitution of the Fe-coordinated aqua ligand and an initial end-on coordination of HP₂O₇³₋ forms [2]. The tri-anionic pyrophosphate species is therefore selectively “trapped” following the switching from a monodentate to a bidentate coordination mode (i.e. linkage isomerism). The elusive intermediate [3] is further stabilized by an additional hydrogen bond, which activates the probe for the subsequent irreversible disassembly reaction and release of the fluorescent signal. The initial binding isomerism of pyrophosphate by the probe is unexpected and illustrates a structural flexible nature of the tetra-dentate Fe³⁺-salen complex in the presence of HP₂O₇³−. This unusual binding property nicely explain the previously observed outstanding selectivity of the probe for HP₂O₇³−. Other analytes such as orthophosphate are however potentially too small and/or lack the chelating capability (e.g. F⁻), or are sterically too demanding (e.g. ATP) for inducing the required structural changes. Alternatively, they might lack the correct geometry and flexibility coupled with appropriate hydrogen donor capability within the metal complex to trigger the disassembly of the metal-analyte complex. This also explains the inability of the di- or tetra-anions of pyrophosphate (H₂P₂O₇²⁻ and P₂O₇⁴⁻) to significantly compete in the overall reaction. It is noteworthy that the mode of initial analyte binding and recognition by the “flexible” metal complex resembles the induced-fit principle in enzymatic catalysis. We anticipate that induced fit binding of anions and molecules to “flexible” metal complexes is not restricted to the combination of pyrophosphate species and Fe-salen complexes. Therefore, we envisage that flexible metal complexes will find interesting applications in analytical chemistry, but also catalysis, medicinal chemistry, material science and related areas in the near future.

Conflicts of interest

The authors declare no competing financial interest.

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