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LETTER

Catalytic hydrolysis of aryl esters by an iron–sulfur cluster

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Iron–sulfur clusters of general formula $[Fe_4S_4(SR)_4]^{2-}$ are synthetic analogs of the active sites of ferredoxins, high potential iron proteins, and some enzymes [1–4]. Catalytic activity of iron–sulfur clusters in chemical and electrochemical redox reactions due to electron or hydride transfer is well known [4, 5]. However, the involvement of an iron–sulfur cluster in aconitase active site [6] indicates their possible catalytic ability in non-oxidative reactions with electrophilic or nucleophilic type of substrate activation.

Iron–sulfur clusters were found to be weak or moderate bases with pK_a values of conjugate acids ranging from 3.9 [7] to 7.4 [8] and 8–11 [9, 10]. This fact prompted us to test their reactivity towards activated esters which undergo hydrolysis in the presence of nucleophilic catalysts of comparable basicity [11, 12]. Here we report the first example of esterolytic activity of an iron–sulfur cluster.

Experimental

The cluster $[Fe_4S_4(SC_6H_5)_4]^{2-}$ (**1**) was prepared according to ref. 13 and isolated as the tetraethylammonium salt. The esters 4-nitrophenyl acetate (NPA) and 2,4-dinitrophenyl acetate (DNPA) were obtained from Fluka and purified by recrystallization from ethyl acetate–acetic anhydride (9/1). 4-Nitrophenyl chloroacetate (NPCA) was prepared according to ref. 14.

The reaction processes were followed by spectrophotometry using the absorbance of respective nitrophenolate anions in a specially constructed cell under strictly anaerobic conditions. Reaction media always contained 0.024 mol dm^{-3} non-ionic surfactant Triton X-100 to increase the cluster solubility [15].

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Results and discussion

Since thiolate anions split nitrophenyl esters rather effectively [16] and they may exist in the cluster **1** solution due to its dissociation [7, 7], special attention was given to exclude this trivial explanation of cluster reactivity.

Cluster **1** is infinitely stable in the pH range 7–10, but decomposes at lower pH values. An approximate pK_a of cluster **1**, evaluated from decomposition rate–pH profile, is 5.5. It reacts with NPA according to a simple second-order rate law, the first in each reactant. The rate constant remains unchangeable up to 400% conversion of NPA with respect to **1**, which is indicative of a catalytic behavior of the cluster.

Figure 1 shows the rate constants for reactions of NPA both with **1** and thiophenol at various pH. In accordance with the above mentioned estimate of the cluster pK_a, the rate of the reaction with **1** is independent of pH in the chosen interval, while the reaction with thiophenol shows an expected rate retardation when pH approaches its pK_a. The cluster is twice as reactive as thiophenolate anion. This fact together with the different pH dependencies and the catalytic mode of action in the case of **1** proves the cluster (and not dissociating thiophenol) to be the reactive species. The reaction rate was found

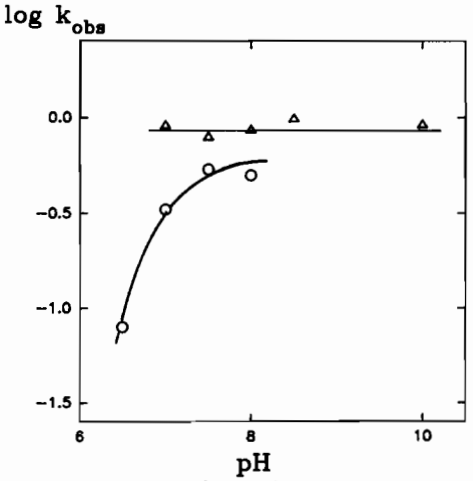

Fig. 1. Log k_{obs} (dm^3 mol^{-1} s^{-1}) vs. pH profiles for the hydrolysis of NPA (△), catalyzed by the cluster **1**, and for the thiolysis of NPA (○) by thiophenol in aqueous micellar solution of Triton X-100 (0.024 mol dm^{-3}) at 25 °C. Concentrations of the ester were from 8×10^{-5} to 4.8×10^{-4} mol dm^{-3} and those of **1** from 2.4×10^{-5} to 1.2×10^{-4} mol dm^{-3}.

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to be the same in D_2O and H_2O solvents which is indicative of a nucleophilic rather than a general base mechanism of catalysis [11, 12].

In the case when a more activated ester (NPCA) was used, the catalytic mode of action was even more evident. When 4×10^{-4} mol dm^{-3} NPCA was hydrolyzed in the presence of 2.6×10^{-5} mol dm^{-3} of **1** at pH 7 more than 1000% of the ester with respect to **1** was converted to products through the cluster-catalyzed path with the rate constant $k_{obs}=22.9$ dm^3 mol^{-1} s^{-1}. The rate constant of thiolysis of NPCA by thiophenol in the same conditions equaled 140 dm^3 mol^{-1} s^{-1}. In this case, therefore, cluster **1** is less reactive than the ligand.

If one assumes a nucleophilic mechanism of cluster catalysis, the existence of two steps, acylation and deacylation, should be expected. We found kinetic evidence in favor of this in the case of hydrolysis of the leaving group activated ester DNPA. A few kinetic curves of the reaction are shown in Fig. 2. A characteristic 'burst' followed by a slow reaction is clearly seen. An initial fast change of optical density is approximately equivalent to the cluster concentration, as should be expected for the acylation step. The kinetics of this first step follows the same second-order rate law as for NPA, the pH-independent rate constant being 16 dm^3 mol^{-1} s^{-1}. The respective rate constant of thiophenolysis of DNPA is 40 dm^3 mol^{-1} s^{-1}.

The kinetics of the second deacylation step was expected to be first order in **1** and zero order in the ester. We found, indeed, first order in **1**, but also approximately first order in DNPA. This anomaly is explicable, although tentatively, on the basis of our preliminary attempts to characterize the iron-containing species after a catalytic run by spectrophotometry and analysis. The results showed cluster **1** to be partly hydrolyzed, losing one or two moles of thiophenol per mole of the cluster, with retention of the iron–sulfur core. In the course of reaction with DNPA, the cluster composition has been changed during the first fast step and then remained constant until complete conversion of the ester. It is quite possible that the 'deacylation' step involves interaction of DNPA with aqua/hydroxo iron-containing complexes resulting from the partial hydrolysis of the starting cluster **1**.

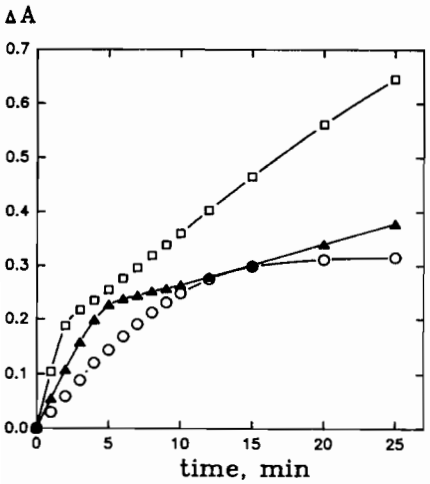

Fig. 2. Variation of absorbance at 405 nm with time for the hydrolysis of DNPA in the presence of **1** (2.6×10^{-5} mol dm^{-3}) at pH 7.0. Concentrations of DNPA: 8×10^{-5} (○), 2.4×10^{-4} (▲), 4.8×10^{-4} (□) mol dm^{-3}; other conditions as in Fig. 1.

Comparing the reactivity of the cluster with that of thiophenolate, we observed the cluster to be less sensitive to the ester structure, in particular to the leaving group. The ratio of the rate constants of thiophenolysis of DNPA and NPA is 80, and that of the cluster-catalyzed hydrolysis is 18. So one may expect clusters of this type to be catalysts for the hydrolysis of non-activated esters.

Comparison of the cluster reactivity towards NPA with those of other nucleophiles of comparable basicity [11, 12] shows it to be a fairly strong nucleophile, even stronger than imidazole with $pK_a=7$.

The results presented above indicate the possible role of the iron–sulfur center in aconitase as a nucleophilic or general base component of the enzymic reaction. Enzyme activity is controlled by dissociation of a group, which assists the reaction by the general base mechanism [6a]. However, this pK_a value is too high for the carboxyl group. The iron–sulfur cluster seems to be a better candidate since the pK_a value found for the structurally related cluster in ferredoxin [17] equals 7.4. Our preliminary results show the cluster $[Fe_4S_4(SCH_2CH_2OH)_4]^{2-}$ with the thiole ligand of comparable to cysteine basicity, to split NPA with pH dependent kinetics exhibiting $pK_a=7.9$.

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