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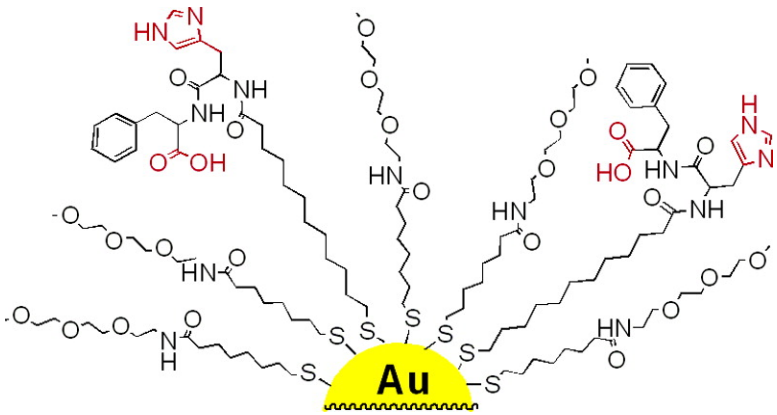

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Carboxylate−Imidazole Cooperativity in Dipeptide-Functionalized Gold Nanoparticles with Esterase-like Activity

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Although the main principles governing enzyme activity are basically understood, the implementation of valid synthetic models still remains a challenging task.[1−6] The contemporaneous control of topology and solvation of the functional groups in a catalytic site is achieved in natural systems with the proper folding of the proteic polymer. Simplified models lack this control, while the synthesis of complex systems is extremely demanding. For this reason chemists have turned their attention to self-assembled catalysts.[5−7] Early and much studied systems are those constituted by functional micelles[6] and liposomes[5,7] where aggregation is driven by hydrophobic effects. Although impressive rate accelerations have been obtained with these aggregation colloids, most of their activity could be explained with the high concentration of reactants at the reaction loci. No clear example of real cooperativity between functional groups has been reported for these systems thus far, likely because of the mobility of the monomers. A possible way to overcome this problem is to anchor the functional groups on a surface.

Systems of this type are easily obtained by passivating a gold nanoparticle with functional thiols (monolayer protected gold clusters, MPCs). Since their synthesis,[8] these self-assembled aggregates have encountered increasing popularity[9−11] due to their peculiar properties. We have shown that their monolayer provides a unique environment[12] that can be exploited to study confinement and clustering effects on the reactivity of functional groups.[13] Hence, they are good candidates for the preparation of catalysts with properties similar to those of natural enzymes.

Thus, we have prepared the dipeptide-functionalized thiol **2** and used it for the passivation of Au nanoparticles[14,15] by exploiting the site-exchange protocol starting from nanoparticles covered with the water-soluble thiol **1**.[16] The resulting functional MPCs **3** composed of a 3:1 ratio thiol **1**:**2**, as determined by ^{1}H NMR, are fully soluble in water[17] and present on the outer surface the terminal carboxylate of the phenyl alanine and the imidazole of histidine. These groups constitute essential elements in the catalytic site of many esterases where they operate in a concerted fashion as general base and general acid in the catalytic process.[18]

We have tested the activity of the new dipeptide-functionalized nanoparticles in the hydrolysis of two activated esters: 2,4-dinitrophenyl butanoate (DNPB) and *Z*-leucine-*p*-nitrophenyl ester (Z-Leu-PNP). The first one presents the advantage of a leaving group with a very low pK_a and hence deprotonates down to pH 4,[19] while the second one is rather lipophilic and thus may take advantage of a tighter binding to the monolayer protecting the gold nanocluster. For the reference catalyst we have used Ac-His-Phe-OH (**4**), the dipeptide that is present on the surface of the functional MPCs but unable to aggregate.

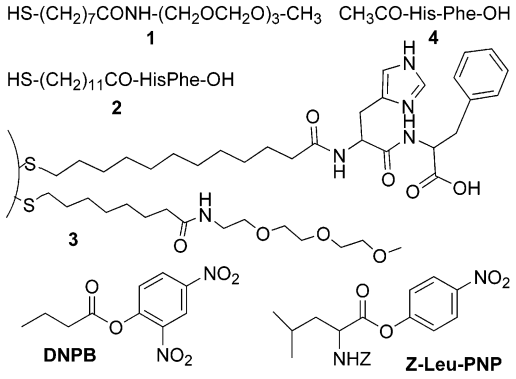

Figure 1 reports the activity against pH of the functional nanoparticles **3** and the monomeric catalyst **4** in the hydrolysis of DNPB as k_{app}^2, the apparent second-order rate constant of the catalyzed process.[20] The two curves show strikingly different profiles. That observed for **4** is the profile expected for a system in which a catalytically relevant nucleophile is generated with pK_a 6.6. This is consistent with the basicity of the imidazole of the histidine. In the case of functional nanoparticle **3** the profile is more complex: a first nucleophilic species is generated with pK_a 4.2, then the curve flattens up to pH 7 where a second nucleophilic species is generated with pK_a 8.1. We assign the first pK_a to the terminal carboxylate of the phenyl alanine and the second one to the imidazole of histidine. This latter is 1.5 units higher than in **4** because of the anionic nature of the surface of the nanoparticle that disfavors the deprotonation of the imidazolium cation. Since the carboxylate contribution to catalysis is completely absent in the monomeric catalyst, the nanoparticle-based system is significantly more active in the low-pH regime (more than 300-fold rate acceleration, see inset to Figure 1), while the rate advantage at pH 10 is only a factor of 35. The high reactivity at acidic pH cannot be attributed to a carboxylate anion acting as a general base or nucleophile alone. The reported[21] pH-independent second-order rate constant for the hydrolysis of similar substrates catalyzed by acetate anions is about 1×10^{-5} M^{-1} s^{-1}. This value is at least 5 orders of magnitude lower than that measured for nanoparticles **3**. This difference can hardly be explained in terms of hydrophobic or medium effects. The interpretation we propose for this anomalous reactivity is that a carboxylate anion acts as a general base activating a water molecule and a protonated imidazole acts as a general acid transferring a proton to the developing tetrahedral intermediate. This mechanism leads, in a straightforward way, to the definition of the

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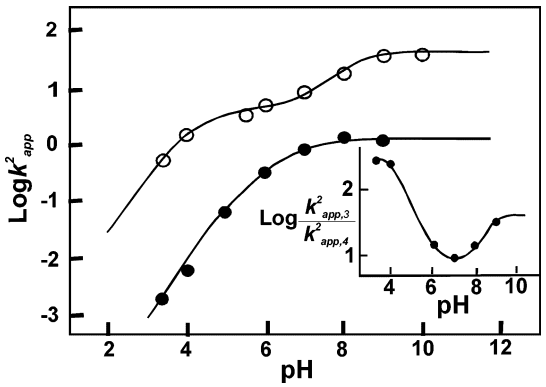

Figure 1. Log of the apparent second-order rate constant against pH for the hydrolysis of DNPB catalyzed by nanoparticles **3** (○) or monomer **4** (●). (Inset) Ratio between the rate constants at the different pHs. Conditions: [buffer] = 10 mM, 25 °C, 10% CH_3OH in H_2O (v/v).

following expression for the second-order rate constant as a function of pH, eq 1:

$$k_{app}{}^2 = k_{coop}{}^2\alpha_{COO-}\alpha_{ImH+} + k_{Im}{}^2\alpha_{Im} \qquad (1)$$

where α_{COO-}, α_{ImH+}, and α_{Im} represent the fraction of ionized carboxylic groups, protonated and unprotonated imidazoles, respectively, at a given pH. $k_{coop}{}^2$ and $k_{Im}{}^2$ are the (pH independent) hydrolytic, second-order rate constants for the cooperative mechanism and for that catalyzed by imidazole acting as a general base or nucleophile, respectively. The solid curve in Figure 1 represents the best fitting of the experimental points using eq 1. The rate constants obtained are 4.2 M^{-1} s^{-1} for $k_{coop}{}^2$ and 38.8 M^{-1} s^{-1} for $k_{Im}{}^2$. An independent proof of the involvement of the imidazole in the mechanism dominant below pH 7 comes from the inhibition (up to 50%, see Supporting Information) of the process at pH 4 and 5.5 by pretreatment of gold nanoparticles **3** with diethylpyrocarbonate and iodoacetamide, both known[22] to suppress the activity of imidazole as a nucleophile and proton donor. Such an inhibition is not observed with the monomeric catalyst **4**. Thus, it is the confinement of the dipeptide on the nanoparticle protecting monolayer that triggers this cooperative mechanism at low pH.

To address the question whether the cooperating units come from the same thiol or from two neighboring thiols we have prepared nanoparticles with different **1**:**2** compositions. Strikingly enough, going from 27 to 18% **2** we found the same activity profile. These results may indicate that cooperativity occurs between carboxylate and imidazolium ions residing on the same thiol or that there is clustering of **2** on the monolayer.[23] This clustering would offset the dilution of **2** in **1**. Clustering of thiols on the monolayer has been reported by Rotello[24] and Stellacci.[25] The reactivity profile observed with the more lipophilic substrate Z-Leu-PNP is similar to that observed with DNPB with the important difference that Z-Leu-PNP binds strongly to the monolayer. Thus, with Z-Leu-PNP we could run kinetics with excess substrate under turnover conditions and obtain a saturation profile analogous to that found in enzymatic catalysis. Analysis of the curve gave $K_M = 50$ μM and $k_{cat} = 1.5 \times 10^{-4}$ s^{-1} at pH 7. At this pH 66% of the activity is due to the cooperative mechanism involving carboxylate and imidazolium ions, while the remaining 33% is that due to the imidazole. The cooperative mechanism is not operative with the monomeric system, and hence no comparison can be made with the nanoparticles. A comparison can, however, be made as for the imidazole catalysis which is present in both systems. It reveals that the reactivity is enhanced 120-fold with the functional MPCs. This reactivity enhancement can be explained fairly well in terms of Brønsted correlation, considering the difference in pK_a value between the imidazole in the aggregate and monomeric catalysts.

In conclusion we have presented the first example of peptide-functionalized gold nanoparticles hydrolytically active against carboxylate esters. The confinement of the catalytic units in the monolayer covering the nanoparticles triggers a cooperative hydrolytic mechanism operative at pH < 7 in which a carboxylate and an imidazolium ion act as general base and general acid, respectively. Such a mechanism is absent with an analogous monomeric dipeptide, and this results in a more than 300-fold rate acceleration of the hydrolytic process at low pH in the presence of the functional nanoparticles. Previously,[13] we had observed cooperativity between *N*-methylimidazoles in functionalized nanoparticles although the system was far less active than the present one. Thus, cooperativity seems to be a rule rather than an exception in functional nanoparticles.

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Supporting Information Available: Synthetic details, TEM analysis of **3**, and inhibition experiments. This material is available free of charge via the Internet at http://pubs.acs.org.

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