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Kinase-Catalyzed Modification of Gold Nanoparticles: A New Approach to Colorimetric Kinase Activity Screening

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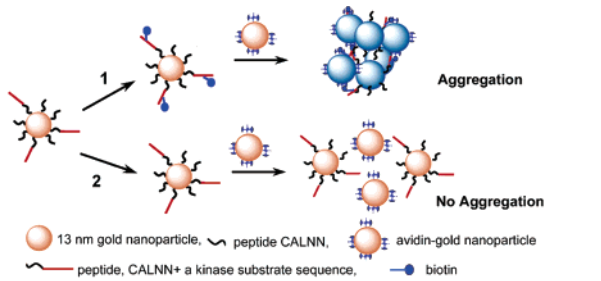
The phosphorylation of proteins by kinases plays vital regulatory roles in most metabolic pathways and in cell communication.[1] Therefore, the identification of kinases, their substrates, and in particular, potential inhibitors is necessary for the understanding of many fundamental biochemical processes, and it is also gaining importance in drug discovery.[1,2] Radiolabeling of the substrate using γ-^{32}P-ATP as cosubstrate is the standard method to monitor kinase activity.[1,2] Nonradioactive alternative methods based on microarray technology are currently emerging.[3] We have recently shown that kinase substrates can be identified by the specific binding of gold nanoparticles to the phosphorylated product immobilized on a microarray.[4] This is achieved by replacing the radiolabeled co-substrate with γ-biotin-ATP, which results in the biotinylation of the substrate, to which nanoparticles modified with avidin then bind with high specificity. These binding events are readily detected by resonance light scattering of nanoparticles.

In this communication, we demonstrate that the same labeling strategy (i.e., biotinylation of the substrate followed by binding to avidin-modified gold nanoparticles) can be employed to develop a colorimetric assay for kinase inhibitors. Importantly, we have now taken advantage of peptide-capped nanoparticles, in which 10% of the peptide ligands carry an extension, which is a substrate for a specific kinase, i.e., we have replaced the natural substrate of the kinase by functionalized gold nanoparticles. Therefore, using γ-biotin-ATP as a cosubstrate, the kinase reaction results in the biotinylation of the gold nanoparticles, one of few examples of a controlled enzymatic modification of an artificial nanoparticle.[5] If the biotinylated particles are then mixed in solution with avidin-modified particles, immediate aggregation of particles occurs due to the specific binding between avidin and biotin.[6] This is manifested in a pronounced color change of the solution from red to blue due to the well-established shift and broadening of the plasmon resonance band in gold nanoparticle aggregates,[7] which has first been exploited to monitor specific biomolecular recognition in Mirkin's and Alivisatos' pioneering work on gold nanoparticle/DNA conjugates.[8] Using a multiwell microplate it is possible to colorimetrically screen in parallel a number of kinase reactions in the presence of potential inhibitors. Change of color indicates kinase-catalyzed biotinylation of substrate particles, while no change of color indicates efficient inhibition of the kinase reaction. This simple detection rationale is illustrated schematically in Scheme 1.

To demonstrate the utility of this approach, we designed and prepared 13-nm gold nanoparticles, which were stabilized by a pentapeptide[9] and additionally modified with two further oligopeptide sequences,[10] which are the known substrates of the cAMP-dependent protein kinase A (PKA) and the calmodulin-dependent kinase II (CaM KII), respectively.[2a] We used two different kinases to demonstrate the potential of this method to screen for inhibitors of several kinases with the same type of nanoparticles. The

Scheme 1. Schematic Representation of Phosphorylation/Biotinylation of Substrate Nanoparticles Followed by Addition of Avidin-Modified Nanoparticles in the Presence and Absence of a Kinase Inhibitor



1, Kinase + biotin-ATP = biotinylation; 2, Kinase + inhibitor + biotin-ATP = no biotinylation

phosphorylation/biotinylation site is in each case the serine residue two units away from the C-terminal amino acid of the sequence. In separate experiments, these particles were incubated with either PKA or CaM KII, both in the presence of γ-biotin-ATP, followed by purification and addition of 13-nm avidin-modified gold nanoparticles. The same experiments were repeated in the presence of well-known kinase inhibitors, H89 for PKA and KN62 for CaM KII.[2a] The solutions were then analyzed by visual inspection and UV−vis spectroscopy. The spectra are shown in Figure 1 (also see Supporting Information, Figures S1−S4). In the presence of an active kinase, the color of the solutions changed from red to blue after addition of the avidin-modified particles, and over a period of several hours complete precipitation of the particles was observed. This is manifested in a broadening and red shift of the UV−vis spectra, which were recorded after resuspension of the precipitates by vigorous shaking. We attribute this behavior to the enzymatic biotinylation of the substrate particles and subsequent aggregation via biotin−avidin binding. To confirm that incubation with kinase indeed resulted in a chemical modification of the ligand

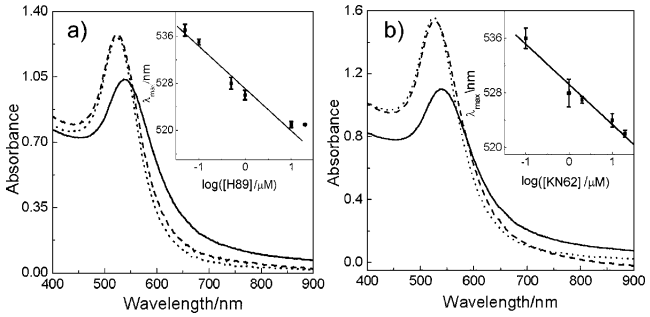


Figure 1. (a) UV−Visible spectra of gold nanoparticle solutions incubated with PKA before (dotted line) and after the addition of avidin-modified nanoparticles in the presence (dashed line) and absence (solid line) of the inhibitor H89. The inset shows the dependence of the peak position on inhibitor concentration. (b) Same as for (a) but for the kinase CaM KII and the corresponding inhibitor KN62.

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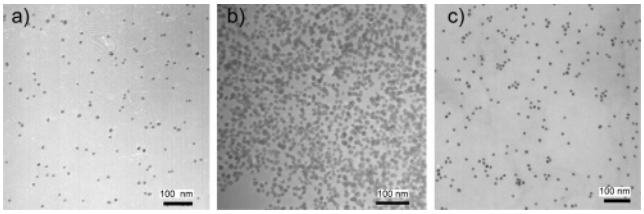

Figure 2. TEM images showing gold nanoparticles incubated with PKA before (a) and after the addition of avidin-modified nanoparticles in the absence (b) and presence (c) of the inhibitor H89.

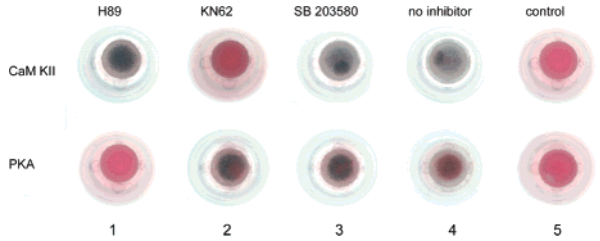

Figure 3. Section of multiwell microplate containing substrate gold nanoparticles and six different combinations (columns 1−3) of three potential inhibitors and two kinases (CaM KII and PKA) after addition of avidin-modified nanoparticles. Control experiments were carried out in the absence of inhibitor (column 4) and in the absence of kinase (column 5). Red indicates no aggregation of particles (i.e., effective inhibition of the respective kinase), while dark blue/gray indicates aggregation (i.e., effective phosphorylation/biotinylation by the respective kinase).

shell, the particles were studied by gel electrophoresis before and after the enzymatic reaction step. The gels show different mobility of the particles before and after the enzymatic reaction clearly indicating their chemical modification (see Supporting Information, Figure S5). As expected, in the absence of kinase, or in the presence of an efficient inhibitor, no detectable color change occurs after addition of the avidin-modified particles, and the solutions are indefinitely stable without showing signs of aggregation. It is noteworthy that at low inhibitor concentrations the red shift of the spectrum is inhibitor concentration-dependent, as shown in the insets of Figure 1. On the basis of this dependency, IC_{50} values of the inhibitors can be estimated (see Supporting Information). This suggests that our method not only has the potential to screen qualitatively for inhibitor activity, but also may be developed further to yield more quantitative information on the efficiency of different inhibitors in comparison with each other. More efficient inhibitors lead to lower levels of biotinylation, which is manifested in the formation of smaller aggregates of particles corresponding to a less pronounced red shift of the spectrum.

The enzymatically induced aggregation of gold nanoparticles in the absence of an inhibitor as well as the inhibition of this process by an efficient inhibitor have been studied also by TEM. The images shown in Figure 2 (also see Figure S6 in Supporting Information) confirm the notion that the particles are linked to each other by biotin−avidin interactions whenever enzymatic biotinylation of the substrate particles occurs. In the presence of a sufficient amount of inhibitor, no significant binding of particles to each other could be observed.

To demonstrate the potential of this method for the parallel screening of a number of inhibitors, we transferred all reactions to a multiwell microplate and evaluated, in a proof of principle scenario, the efficiencies of three different inhibitors (H89, KN62, and SB203508) for the two kinases (PKA and CaM KII) simultaneously. The result of this experiment is shown in Figure 3. Visual inspection of the different solutions in the multiwell microplate immediately yields valuable information on the efficiency of the three compounds as inhibitors for each of the two different kinases. A red solution indicates enzyme inhibition, while a blue solution means that enzymatic biotinylation of the substrate nanoparticles has occurred. A simple result that can be directly read off Figure 3 is that H89 inhibits PKA but not CaM KII, KN62 inhibits CaM KII but not PKA, and SB 203580 inhibits neither of the two kinases. This is in accordance with the known inhibitor activities of these three compounds.[2a]

In conclusion, the use of specifically designed, peptide-stabilized gold nanoparticles as artificial substrates for kinases has been demonstrated. Owing to the unique optical properties of these particles, a very simple colorimetric protocol for the evaluation of kinase activity and inhibition was developed. This may have important implications for the future use of nanoparticle-based technologies in drug discovery.

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Supporting Information Available: Details of UV−visible spectra studies, agarose gel electrophoresis of the phosphorylation/biotinylation of gold nanoparticles, TEM of gold nanoparticles incubated with CaM KII after the addition of avidin-modified nanoparticles in the absence and presence of KN62 and experimental preparation, and complete citation for ref 2d. This material is available free of charge via the Internet at http://pubs.acs.org.

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(10) Peptide sequences: pentapeptide stabilizer, CALNN, and substrate oligopeptides, CALNNAALRRASLG, CALNNAAKKLNRTLSVA.

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