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The Different Fluorescent Response of Quin 2 to the Binding of Ca^{2+} and La^{3+} and its Application

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Abstract: The fluorescence spectra of Quin 2, (2-[(2-bis-[carboxymethyl] amino-5-methylphenoxy) methyl]-6-methoxy-8-bis [carboxymethyl] aminoquiniline), a Ca^{2+} probe, were investigated upon incubation with Ca^{2+} or La^{3+}. The results showed that binding of La^{3+} to Quin 2 resulted in different fluorescent spectrum from that of Ca^{2+}. Based on this observation, a fluorescent method was developed for simultaneously determination of the dissociation rates of Ca^{2+} and La^{3+} from a Ca-La- calmodulin complex (Ca_2La_2CaM).

Keywords: Quin 2, fluorescence, Ca^{2+}, La^{3+}, calmodulin.

For years, many biological effects of lanthanides were considered to be related with calcium. It is well known that, due to the similarity between Ca^{2+} and lanthanide ions in coordination chemistry, lanthanides can compete with and/or substitute Ca^{2+} from calcium binding proteins and also have some influences on intracellular Ca^{2+} homeostasis[1,2]. So in a biological system containing both Ca^{2+} and lanthanide ions, a convenient method of identification of Ca^{2+} and lanthanide ions in a mild condition would be useful to measure the concentration and clarify the status of these two types of ions. In the present work, we reported the great differences between the fluorescent spectra of Ca-Quin 2 complex and that of La-Quin 2 complex. Based on such differences, the dissociation rates of Ca^{2+} and La^{3+} from Ca_2La_2CaM complex were measured simultaneously in a fluorescence stopped-flow experiment.

Experimental

Fluorescence experiments were performed on RF5301 fluorometer at room temperature in a Fluorescence Buffer containing 20 mmol/L Hepes, pH 7.0, 100 mmol/L KCl. All the solution used was passed through a Chelex 100 column to remove possible contamination of high valence metal ions. The concentration of Quin 2, Ca-Quin 2 and La-Quin 2 were 10 μmol/L. To record the excitation spectra from 300 nm to 400 nm,

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490 nm was used as emission wavelength, and 336 nm was used as excitation wavelength for the emission spectra (450 nm to 550 nm) likewise. The slit widths were 10 nm for all fluorescence measurements unless indicated.

Fluorescence stopped-flow experiments were performed on Cary Eclipse fluorospectrometer equipped with a SPF-20 stopped-flow accessory at room temperature. All the stopped-flow experiments were performed in the fluorescence buffer. Mixture of protein solution (containing 5 μmol/L of Ca_2La_2CaM complexes) and chelator solution (100 μmol/L Quin 2) was conducted using two manual syringe pumps. The excitation wavelength was 336 nm with a slit width of 2.5 nm, and the emission wavelength was 490 nm. Data were fitted by double exponential function using a MicroCal Origin™ program.

Results and Discussion

Quin 2 is the first generation of Ca^{2+} probe. As a derivative from EGTA, Quin 2 forms complexes with many high valence metal ions at the molar ratio of 1:1. As in many cases, La^{3+}, the closest analog among Ln^{3+} to calcium ion, can tightly bind to Quin 2 and change its fluorescence spectrum. The fluorescence spectra of Quin 2, Ca-Quin 2 and La-Quin 2 were shown in **Figure 1**. It is obvious that the responses of Quin 2 to the binding of Ca^{2+} and that of La^{3+} are quite different. As described previously[3], the binding of Ca^{2+} to Quin 2 increases the fluorescence intensity about ten folds, along with a blue shift of the maximum excitation wavelength from 354 nm to 332 nm as well as a small red shift of the maximum emission wavelength from 487 nm to 494 nm. However, the binding of La^{3+} to Quin 2 gives rise to the great decrease of fluorescence intensity. The blue shift of the maximum excitation wavelength is close to that caused by Ca^{2+}, but red shift of the emission spectrum is larger (from 487 nm to 513 nm). So in a system containing both Ca^{2+} and La^{3+}, discrimination between Ca^{2+} and La^{3+} could be accomplished by selecting a suitable excitation wavelength: since the excitation curve of Ca-Quin 2 complexes joins with the free ligand at 355 nm, 355 nm could be to used to monitor formation of La^{3+}-Quin 2 complexes because binding of La^{3+} decrease the fluorescence intensity but Ca^{2+} does not; 332 nm can be used for determination of Ca^{2+} and La^{3+} together because at which excitation wavelength Ca^{2+} increase the florescence intensity but La^{3+} decrease it. It should be mentioned that such properties of Quin 2 are likely to be unique among current Ca^{2+} fluorescence probes, such as Fura 2 and Indo 1. These Ca^{2+} probes show the similar fluorescence spectra when they bind to Ca^{2+} or La^{3+} [4,5]. The mechanism of different response of Quin 2 to La^{3+} and Ca^{2+} might be related with the structural differences between Ca-Quin 2 and La-Quin 2, and coordination water molecules might be involved in quenching the fluorescence of the La^{3+}-Quin 2 complexes.

The new method facilitated the studies of kinetic behaviors of a Ca-La- calmodulin complex (Ca_2La_2CaM). **Figure 2** shows the time course of dissociation of Ca^{2+} and La^{3+} from the Ca_2La_2CaM upon adding Quin-2 monitored at the $\lambda_{ex/em}$ = 332/490 nm on a stopped-flow fluorometer. In the above experiment, Quin 2 was a chelator as well as an indicator. Calmodulin (CaM) is a calcium binding protein that plays very important role

Figure 1 Excitation (left) and emission (right) spectra of Quin 2 (dash line), Ca-Quin 2 (solid line) and La-Quin 2 (dot line)

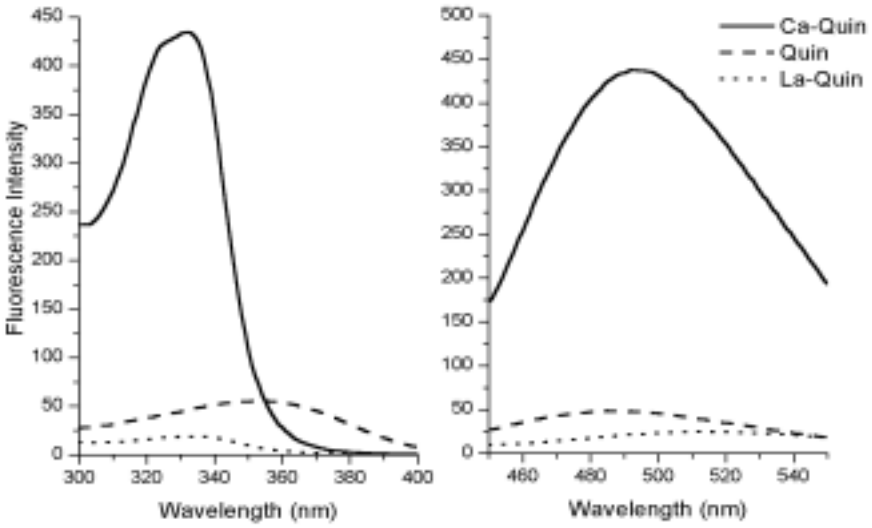
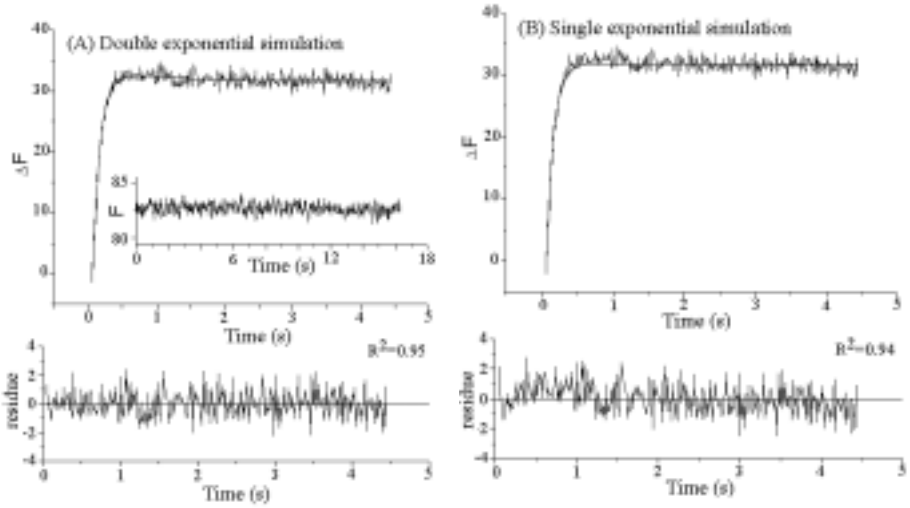


Figure 2 The time course of dissociation of Ca2+ and La3+ from a Ca-La-calmodulin complex (Ca2La2CaM) monitored by the stopped-flow fluorescence using Quin-2 (The λex/em = 332/490 nm)



The results from double exponential simulation and single exponential simulation are compared. The insert in (A) shows the time course of Quin 2 alone.

in intracellular calcium signal transduction. In CaM, there are four Ca^{2+} binding sites and La^{3+} was found to bind to these sites with high binding affinity[6].

We had observed in NMR studies (unpublished data) that Ca^{2+} and La^{3+} formed hybrid complexes with CaM by two Ca^{2+} binding to the C-terminal and two La^{3+} binding to the N-terminal. It can be seen from **Figure 2** that releasing of metal ions from Ca_2La_2CaM was a two-phasic process: a faster process with a large increase in fluorescence and a slower fluorescence decrease process. As described above, the faster fluorescence increase should be corresponding to the release course of Ca^{2+}, and accordingly, the slower one corresponding to the release of La^{3+}. By fitting the data in **Figure 2** to the double exponential function using a MicroCal Origin™ program (one can find that double exponential function simulation is better than the single exponential function simulation in **Figure 2**), the dissociation rate constants were determined to be $8.1s^{-1}$ for Ca^{2+} and $1.2s^{-1}$ for La^{3+}, respectively. These results are in good consistence with those reported previously[7,8].

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