A Fluorescence Stopped-flow Kinetic Study of the Displacement of 2-[(2-Bis[carboxymethyl]amino-5-methylphenoxy)methyl]-6-methoxy-8-bis[carboxymethyl]aminoquinoline (quin2) from its Ca^{2+}, Pr^{3+}, Tb^{3+}, Dy^{3+}, and Yb^{3+} Complexes by Ethylenedinitrilotetraacetate (edta) in Aqueous Solution

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Abstract

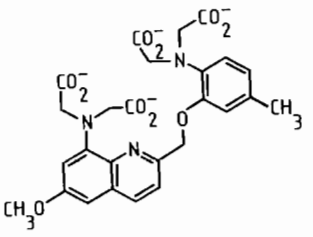
In aqueous solution at pH 7.00 the observed first order rate constant, k_{obs} (298.2 K), for the displacement of quin2 from several of its metal complexes by edta in excess concentration is of the form:

$k_{obs} = k_1 + k_2[edta]$

For the Ca^{2+}, Pr^{3+}, Tb^{3+}, Dy^{3+}, and Yb^{3+} quin2 complexes $k_1 = (4.48 \pm 0.04) \times 10^1$, $(2.87 \pm 0.51) \times 10^{-3}$, $(1.99 \pm 1.21) \times 10^{-4}$, $(1.27 \pm 0.89) \times 10^{-4}$, and $(9.02 \pm 1.56) \times 10^{-6}$ s^{-1} respectively; and $k_2 = (1.41 \pm 0.03) \times 10^3$, $(9.70 \pm 0.80) \times 10^{-1}$, $(8.48 \pm 0.61) \times 10^{-2}$, $(7.40 \pm 0.52) \times 10^{-2}$, and $(2.40 \pm 0.12) \times 10^{-3}$ dm^3 mol^{-1} s^{-1}. The origins in the lability differences are discussed, and mechanistic interpretations of the data are presented.

Introduction

The tetracarboxylate ligand 2-[(2-bis[carboxymethyl]amino-5-methylphenoxy)methyl]-6-methoxy-8-bis[carboxymethyl]aminoquinoline, usually referred to as quin2, has the structure shown below [1].



Quin2 exhibits a fluorescence which is greatly increased over the range 450–600 nm (excitation at 334 nm) on the rapid formation of a 1:1 complex with Ca^{2+}, characterised by an apparent stability constant of 8.7×10^6 dm^3 mol^{-1}, and in consequence is used as a specific calcium indicator [2–4]. In the course of a more extensive study of trivalent lanthanide ion chemistry we have observed that quin2 is also complexed by these ions. Some trivalent lanthanides specifically displace Ca^{2+} in biological systems, and in consequence have been used as paramagnetic probes in NMR studies [5]. It is therefore of interest to utilise our observations on the complexation of trivalent lanthanide ions by quin2 to make direct comparisons of the relative labilities of Ca^{2+} and the trivalent lanthanides in the same complexes, and of lability variations among the trivalent lanthanides. The trivalent lanthanides chosen for study: Pr^{3+}, Dy^{3+}, Tb^{3+}, and Yb^{3+} are spread across the lanthanide contraction, and accordingly encompass the range of trivalent lanthanide ligand substitution characteristics [6].

Experimental

The fluorescent species 2-[(2-bis[carboxymethyl]-amino-5-methylphenoxy)methyl]-6-methoxy-8-bis-[carboxymethyl]aminoquinoline, quin2 (Sigma) slowly develops a faint pink colouration, presumably due to decomposition, in water under normal laboratory conditions over a period of several weeks. To eliminate this effect stock solutions were prepared immediately prior to use, and were stored in plastic containers in a refrigerator. All solutions studied were 2.0×10^{-2} mol dm^{-3} in sodium piperazine-*N*,*N'*-bis(2-ethane-sulfonate), pipes buffer (Calbiochem) and were adjusted to pH 7.00 with sodium hydroxide (B.D.H. Aristar). The metal salts used in the solution preparations were $CaCl_2 \cdot 2H_2O$ (B.D.H. Analar) and $Pr(ClO_4)_3 \cdot 9H_2O$, $Tb(ClO_4)_3 \cdot 9H_2O$, $Dy(ClO_4)_3 \cdot 9H_2O$, and $Yb(ClO_4)_3 \cdot 9H_2O$, prepared from the appropriate oxides (Fluka). Edta solutions were prepared from disodiumethylenedinitrilotetraacetate (Univar Analytical Reagent). All solutions were prepared in doubly distilled water.

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All fluorescence spectra were run on a Perkin Elmer 3000 fluorescence spectrometer. Kinetic studies of the Ca^{2+}, Pr^{3+}, Tb^{3+} and Dy^{3+} systems were carried out on a stopped-flow fluorescence spectrometer similar to that described elsewhere [7], and constructed in our laboratories. The displacement of quin2 from its Ca^{2+} and trivalent lanthanide complexes is characterised by a decrease and an increase in fluorescence at 490 nm respectively (excitation at 334 nm). Each stopped-flow kinetic trace was digitised as 1024 points using a Datalab DL905 transient recorder and stored on magnetic tape. For each solution six to ten traces were recorded, prior to signal averaging and kinetic analysis using a Computer Data Products Spectrum 11 computer. A small and rapid decrease in the fluorescence of quin2 was observed upon mixing with edta in the absence of added metal ions under the same conditions as those employed for the kinetic studies of the quin2 metal complexes. A stopped-flow study of this phenomenon yielded rate constants in the same range as those observed for the Ca^{2+} system, and it was concluded that a very low level of Ca^{2+} contamination was present. However, as in all cases the metal ion concentrations were in considerable excess over the quin2 concentration in the stopped-flow studies of the quin2 metal complexes, and the reaction rates of the trivalent lanthanide systems were very much slower than those of the Ca^{2+} system, the presence of this contaminant had no significant effect on the kinetic studies reported herein. The kinetics of the least labile system, Yb^{3+}, were studied using the Perkin Elmer 3000 fluorescence spectrometer by conventional methods.

The above kinetic studies were carried out in the [edta] range 2.50×10^{-3} to 3.50×10^{-2} mol dm^{-3} at pH 7.00 and at 298.2 K. For the Ca^{2+} system the total [Ca^{2+}] and [quin2] were 2.51×10^{-5} and 1.24×10^{-5} mol dm^{-3} respectively; and in the Pr^{3+}, Dy^{3+}, Tb^{3+}, and Yb^{3+} systems the total metal ion concentrations were (4.32, 4.96, 4.58, and 4.30) $\times 10^{-5}$ mol dm^{-3} respectively, with total [quin2] = 1.25×10^{-5} mol dm^{-3} in each case. All solutions were 2.0×10^{-2} mol dm^{-3} in pipes buffer.

Results and Discussion

In contrast to the Ca^{2+}–quin2 complex, which exhibits an increased fluorescence over quin2 alone in the 450–600 nm range with excitation at 334 nm, the Pr^{3+}–, Tb^{3+}–, Dy^{3+}–, and Yb^{3+}–quin2 complexes exhibit a greatly decreased fluorescence, as illustrated by the Pr^{3+}–quin2 complex in Fig. 1. In this system fluorescence decreases systematically with a total quin2 concentration invariant at

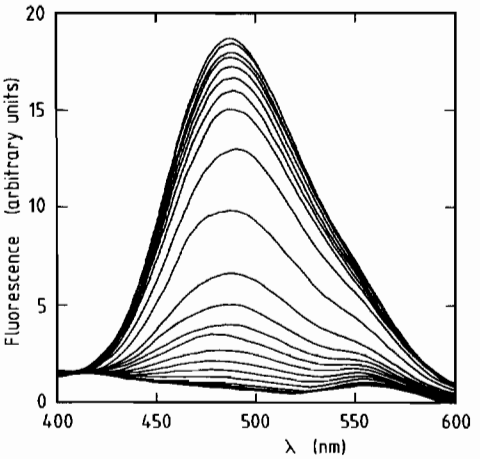


Fig. 1. The variation of the fluorescence spectrum of quin2 with [$Pr(ClO_4)_3$] at pH 7.00 and 298.2 K in an aqueous solution of 2.0×10^{-2} mol dm^{-3} pipes buffer. The total [quin2] = 2.039×10^{-5} mol dm^{-3} and the total [Pr^{3+}] increases in the order 0, (0.06663, 0.1511, 0.2213, 0.3152, 0.4146, 0.5135, 0.6257, 0.7198, 0.8349, 0.9435, 1.032, 1.128, 1.222, 1.319, 1.410, 1.495, 1.587, 1.681, 1.771, 1.860, 1.954, 2.053, and 2.148) $\times 10^{-5}$ mol dm^{-3} as the maximum in successive fluorescence spectra decreases. The excitation wavelength was 334 nm.

2.039×10^{-5} mol dm^{-3}, as the total Pr^{3+} concentration increases from 0 to 2.053×10^{-5} mol dm^{-3}. The fluorescence thereafter remains constant, consistent with the formation of a 1:1 Pr^{3+}–quin2 complex alone when the total Pr^{3+} concentration is equal to or greater than the total quin2 concentration. However, this fluorescence variation is inconsistent with the formation of a 1:1 complex alone at the lower total Pr^{3+} concentrations, and a second species of a 1:2 Pr^{3+} to quin2 ratio appears to be a possibility. Similar observations were made for the Tb^{3+}, Dy^{3+}, and Yb^{3+} systems. The variation of the fluorescence of solutions of Ca^{2+} and quin2, covering a concentration range similar to that employed in the trivalent lanthanide studies, is consistent with a 1:1 Ca^{2+}–quin2 complex being the predominant species in solution over the concentration range studied. This difference between the Ca^{2+} system and the trivalent lanthanide systems may be a reflection of the lower charge of Ca^{2+} by comparison to that of the trivalent lanthanides. The major aim of the fluorescence equilibrium studies was to establish, as a preliminary to kinetic studies, the stoichiometry of the quin2 complex formed when the total trivalent lanthanide ion concentration was equal to or exceeded the total quin2 concentration, and accordingly the nature of the second complex existing at lower lanthanide ion concentrations was not further investigated. (The highest pK_a of quin2 is <7 and therefore, under the conditions of this study, quin2 exists

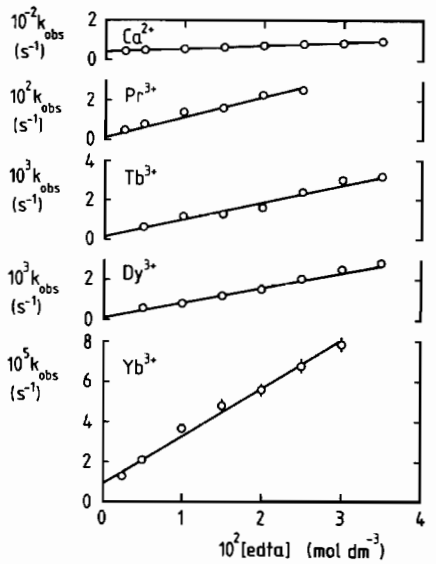


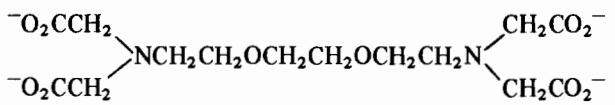
Fig. 2. The variation of k_{obs} for the displacement of quin2 from its metal complexes with [edta] at pH 7.00 and at 298.2 K. For the Ca^{2+} system the total [Ca^{2+}] and [quin2] were 2.510×10^{-5} and 1.241×10^{-5} mol dm^{-3} respectively; and in the Pr^{3+}, Dy^{3+}, Tb^{3+}, and Yb^{3+} systems the total metal ion concentrations were (4.316, 4.962, 4.576, and 4.300) $\times 10^{-5}$ mol dm^{-3} respectively, with total [quin2] = 1.249×10^{-5} mol dm^{-3} in each case. All solutions were 2.0×10^{-2} mol dm^{-3} in pipes buffer. The solid lines represent the least squares fit of these data to eqn. (1).

predominantly as a tetra-negative anion [2]. The highest pK_a of edta is 10, and accordingly it exists predominantly as a tri-negative anion under the conditions of this study [8].)

It is seen from Fig. 2 that the variation of the observed first order rate constant for the displacement of quin2 by edta in excess concentration from the five metal ions studied, k_{obs}, is characterised by:

$$k_{obs} = k_1 + k_2[\text{edta}] \qquad (1)$$

Although an X-ray determination of the structure of a quin2 metal complex has not been reported, it seems probable that quin2 acts as an octadentate ligand, when comparisons are made with the denticities of similar ligands. Thus egta^{4-}, 3,12-bis(carboxymethyl)-6,9-dioxa-3,12-diazatetradecanoate:



which possesses, in addition to its two iminodiacetate groups, two potentially coordinating oxygen atoms in its backbone, acts as an octadentate ligand in $[Ca(egta)]^{2-}$, in which Ca^{2+} is eight coordinated [9]. Hence, the displacement of quin2 from a metal complex will occur in at least eight sequential steps.

Kinetic studies of the displacement of similar polyaminocarboxylate ligands from metal complexes have seldom detected more than two such steps, and it is generally considered that two major steps in the displacement process are the sequential displacement of the two iminodiacetate groups. Thus a simplified explanation of the form of rate law (1) for the displacement of quin2 involves a fast step in which one iminodiacetate group of quin2 detaches from the metal ion, M^{m+}. This is followed by either the slower detachment of the second iminodiacetate group (k_1) to give solvated M^{m+} which is subsequently complexed by edta; or the slower displacement of quin2 by edta (k_2), which proceeds through an intermediate in which both quin2 and edta are bound to M^{m+}, probably through an iminodiacetate group in each case, as shown in Fig. 3. This mechanism is similar to that proposed for the exchange of edta on the Ca^{2+}–edta complex [8], which is characterised by first and second order rate terms analogous to those observed in the rate laws observed for the displacement of quin2 from the five metal ions considered in this study.

The Ca^{2+}–complex is several orders of magnitude more labile than the trivalent lanthanide–quin2 complexes, which suggests that the lower surface charge density of Ca^{2+} is a major cause of the greater lability. In the case of the four trivalent lanthanide–quin2 complexes the magnitudes of both k_1 and k_2 increase as the ionic radius [10] decreases from Pr^{3+} to Yb^{3+} (Table I), in a similar manner to that observed for the displacement of edta and similar ligands from trivalent lanthanide complexes [5, 11, 12]. As these magnitudes increase concommitantly with increase in the ionic radius [10] (Table I) it seems that a decrease in the surface charge density

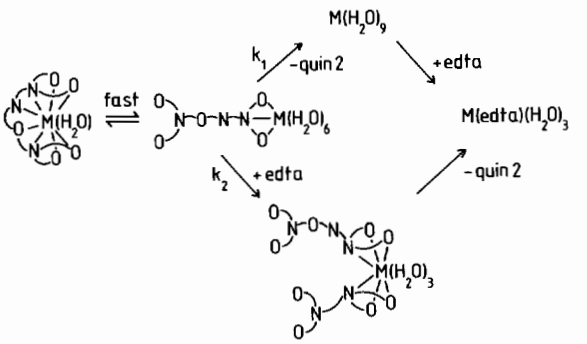


Fig. 3. The proposed mechanism for the displacement of quin2 from its metal complex by edta. In the representation of these ligands only the potential coordinating atoms are shown. Quin2 and edta are shown as octa- and hexadentate ligands respectively, and for simplicity the aromatic nitrogen and ether oxygen of the quin2 backbone are only shown as coordinating atoms when both iminodiacetate groups are coordinated. The metal centre, M, is shown as nine coordinated in all complexes, and all charges are omitted.

TABLE I. Rate Constants[a] for the Displacement of quin2 from its Metal Complexes by edta in Aqueous Solution at pH 7.00 and 298.2 K

Metal	$r(M^{m+})$[b] (pm)	k_1 (s^{-1})	k_2 ($dm^3\ mol^{-1}\ s^{-1}$)
Ca^{2+}	118	$(4.48 \pm 0.04) \times 10^{1}$	$(1.41 \pm 0.03) \times 10^{3}$
Pr^{3+}	117.9	$(2.87 \pm 0.51) \times 10^{-3}$	$(9.70 \pm 0.08) \times 10^{-1}$
Tb^{3+}	109.5	$(1.99 \pm 1.21) \times 10^{-4}$	$(8.48 \pm 0.06) \times 10^{-2}$
Dy^{3+}	108.3	$(1.27 \pm 0.89) \times 10^{-4}$	$(7.40 \pm 0.52) \times 10^{-2}$
Yb^{3+}	104.2	$(9.02 \pm 1.56) \times 10^{-6}$	$(2.40 \pm 0.12) \times 10^{-3}$

[a]The quoted errors represent one standard deviation for the least squares fit of the data to eqn. (1). [b]Nine coordinate ionic radii from [10].

of the trivalent lanthanide ion favours an increase in the quin2 displacement rate, coincident with a decrease in the metal ion–ligand dipole interaction. However, if steric strain in coordinated quin2 increases as the ionic radius decreases, a similar trend in the rate of quin2 displacement should also result. The coordination numbers of the quin2 complexes and the intermediate species shown in Fig. 3 are not established. However, the stoichiometry $[M(H_2O)_9]^{3+}$ is well established for the hydrated trivalent lanthanide ions in the solid state, and as coordination numbers of 8 and 10 are also reported for other trivalent lanthanide complexes, it is probable that the coordination numbers of the species shown in Fig. 3 are encompassed by a value of 9 ± 1 for the five metal ions considered [6].

The variation of the ratio k_1/k_2 is within the range 0.0017–0.0038 for the trivalent lanthanide–quin2 complexes, which suggests that the same factors affect the variation of k_1 and k_2, and is consistent with both the k_1 and k_2 paths proceeding through the rapidly produced intermediate species, in which quin2 is attached by one iminodiacetate group as shown in Fig. 3. The slower displacement of quin2 from this species by water is characterised by k_1; and the coordination of the incoming edta to this species through one iminodiacetate group is the rate determining step for the displacement of quin2 by edta characterised by k_2.

The biological implication to be drawn from this study is that the substitution of trivalent lanthanides for Ca^{2+}, in biological processes, for which the off rate of the metal ion is a critical parameter, will result in a drastic slowing of the biological rate process.

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