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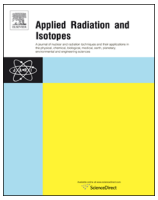

Reversed phase free ion selective radiotracer extraction (RP-FISRE): A new tool to assess the dynamic stabilities of metal (-organic) complexes, for complex half-lives spanning six orders of magnitude

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HIGHLIGHTS

- A new Radiotracer-based RP-FISRE technique developed.
- First-ever data on k_d's for ^{177}Lu-DOTAtate.
- Rate constants k_d of the dissociation of Lu-DOTA-Octreotate (ML, MHL, MH_2L forms) determined as $k_{ML}=1.1\ 10^{-8}\ s^{-1}$, $k_{MHL}=7.4\ 10^{-6}\ s^{-1}$, $k_{MH2L}=1.1\times10^{-4}\ s^{-1}$.

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ABSTRACT

This paper introduces reversed phase free ion selective radiotracer extraction (RP-FISRE) as a new tool to assess the stability of metal complexes, as illustrated by the assessment of the stability of [^{177}Lu]Lu-DOTA-octreotate. To this end, the TUDelft-developed FISRE, where the released metal is column-retained and the complex eluted, was changed into RP-FISRE, where the complex is column-retained and the released metal is eluted. This change in the approach allows for studies to be performed with high stability complexes. This paper presents RP-FISRE, the strength of the radiotracer approach, and the first-ever k_d data on the release of ^{177}Lu from [^{177}Lu]Lu-DOTA-octreotate.

1. Introduction

1.1. Significance of stability kinetics

Metal complexes are important in e.g. biological systems, in redox and enzyme action, to prevent precipitation, toxicity or adsorption, and are used in a large variety of applications, such as in catalysis, material synthesis, photochemistry and medicine. In medicine, they are used for diagnostics as well as for therapy. Well known examples are GdDOTA as a contrast agent in MRI (Herborn et al., 2007), CrEDTA for evaluation of kidney function (Groth et al., 1989), the cytostatic Cisplatin (Reedijk, 2009), and the more recently applied [^{177}Lu]Lu-DOTA-Octreotate for peptide receptor radionuclide therapy (PRRT) of somatostatin receptor-positive tumor cells (Pool et al., 2010). The stability of these metal complexes is of great importance, both because some of the metals used are highly toxic as free ions, and because the efficacy of therapy or diagnostics depends on the ligand-dependent targeted distribution of the metal.

Thermodynamic stability constants are often used as a measure of stability. In clinical applications however, knowledge of the *kinetic stability* is much more relevant because the thermodynamic stability refers to an equilibrium situation which does not exist after injection of a complex into the bloodstream. After injection free metal and free ligand will be diluted in the body fluids, (re-) association will not occur and the dissociation will be determined by dissociation kinetics.

Although the thermodynamic stability constant and the kinetic dissociation rate constant are related, a high thermodynamic stability constant is no guarantee for a low kinetic dissociation rate constant (Kotek et al., 2006). To determine the dissociation

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kinetics of a metal complex not only a suitable method is needed, the use of an appropriate model to interpret results is as important. In the present paper a method is described and a model is proposed which can be applied to determine the rate constants involved in dissociation also permitting the simulation of release of metal from metal complexes.

For synthesis of metal complexes, thermodynamic constants such as stability constants and protonation constants which refer to an equilibrium situation can be used to predict the endpoint concentrations of a complex and its protonated complex species in equilibrium. For some complexes however the equilibration time needed is found to be extremely long. In that case the use of kinetic rate constants for simulation of synthesis or the behavior at several pH's is more convenient: the species concentrations can be predicted over time.

1.2. Existing methods for measurement of stability kinetics

1.2.1. Measurement of complex, metal or ligand

To measure dissociation, either the increase of free metal ions or the decrease of complex over time can be determined. For very stable complexes, measurement of metal released will be more accurate than measurement of the decrease of complex: the latter will be very small compared to the total amount of complex in a time-limited period of observation. An increase of free metal from 1% to 2% for example will be more accurately determined than a decrease of complex from 99% to 98%. For less stable complexes, measurement of the change over time of the amount of complex is more accurate. Sometimes the decrease of one complex is measured indirectly by the formation of another complex either by addition of a second metal (metal exchange) (Cacheris et al., 1990; Kotek et al., 2006; Toth et al., 1994; Kumar et al., 1993) or by the addition of a second ligand (ligand exchange) (Gerringa et al., 2007; van Leeuwen and Town, 2005). The determination of dissociation of one complex by the formation of another complex is a possible reason for erroneous results because dissociation of the secondary complex cannot be ruled out and the formation of the secondary complex can be slow. Here, if dissociation rates found do not depend on the concentration of the added metal or ligand, dissociation is supposed to be the rate-controlling step in the exchange reaction. As an example: the dissociation of GdDOTA could be measured by the detection of the UV signal from $EuDOTA^-$ formed by metal exchange (Toth et al., 1994). Metal exchange was also used to determine the dissociation kinetics of Gd, Y and Cu complexes with DO3A by the formation of As(III)do3a (Cai and Kaden, 1994). The dissociation of GdDTPA complexes was determined by the formation of CuDTPA complexes after addition of an excess of Cu ions (Cacheris et al., 1990).

1.2.2. Detection

Methods frequently used for detection of the decrease of complexes are UV-absorption (Brucher et al., 1987; Cai and Kaden, 1994; Chang and Liu, 2000; Taborsky et al., 2007; Toth et al., 1994) and laser induced luminescence spectroscopy (Piskula et al., 2007; Taborsky et al., 2005; Wu and Horrocks, 1995). Other methods are relaxometry (Broan et al., 1991) or voltammetry (van Leeuwen and Town, 2005). An alternative method of detection is the measurement of the radioactivity of free metal ions or complex in case of a radiolabeled complex (Denkova, 2006; Jurkin et al., 2007; Pulukkody et al., 1993; Stimmel and Kull, 1998; Wang et al., 1992; van Doornmalen et al., 2000; Nayak et al., 2009; van Doornmalen et al., 2002).

Concentrations needed for UV detection or relaxometry for LnDOTA complexes are in the order of millimoles per liter. This is high compared to the limit of detection of radionuclides which is dependent on the radionuclide used but can be in the order of picomoles per liter. Measurements can be performed on-line or off-line: off-line can be regarded as generally more sensitive, but because of the delayed measurement, off line detection is possible only if the process of dissociation is “stopped”, which is often accomplished by separation of free metal and complex/free ligand. Concentrations needed for voltammetry are in the order of nanomoles per liter. The high concentrations needed for UV and relaxometry can be a disadvantage if the complex used is costly, when concentrations used in medical or other practical applications are small or when problems exist with respect to solubility.

For voltammetry, ligand exchange is used to achieve adsorptive accumulation of the complex formed from the released metal ions and the secondary ligand at the electrode surface. For UV or luminescence spectroscopy some complexes can be detected in the presence of free metal ions and free ligand because the latter do not show significant UV absorption at the wavelengths used. The dissociation of $EuDOTA^-$ and $CeDOTA^-/CeH_2DOTA^+$ was measured by detection of the UV-absorption of the complexes at 253–267 nm (Toth et al., 1994) and ∼319 nm/∼297 nm respectively (Brucher et al., 1987; Burai et al., 1998; Chang and Liu, 2000). The dissociation of $EuDOTA^-$ can also be measured by laser induced luminescence.

In the case of radiolabeled complexes or complexes of which no distinction can be made between free ligand and complex when measured by UV, the free metal ions must be separated from the complex to measure free metal only or complex only. Separation of free metal ions from complex or free ligand after preset time intervals is mostly achieved by a cation exchange material which is either added to the complex solution (Wang et al., 1992) or used in a column (Denkova, 2006; Jurkin et al., 2007; van Doornmalen et al., 2002). The column chromatographic method for separation of free metal and complex is also referred to as free ion selective radiotracer extraction (FISRE) which was developed in Delft by van Doornmalen et al.,2000, van Doornmalen et al., 2001 and van Doornmalen et al., 2002. Alternative methods used for separation are anion exchange chromatography of negatively charged secondary complexes (Broan et al., 1991; Pulukkody et al., 1993), TLC (Stimmel and Kull, 1998), capillary electrophoresis (Zhu and Lever, 2002) or dialysis (Nayak et al., 2009).

1.2.3. Dealing with association

To measure dissociation only, association of dissociated metal ions with free ligand should be prevented. If association is not stopped but is ignored in the interpretation, this may result in an underestimation of dissociation. When association is not prevented, this reaction should be included in the interpretation which will complicate the interpretation of results obtained (Denkova, 2006; van Doornmalen et al., 2002). In their experiments, measurements were performed in which a radioactive metal and a ligand were mixed in a solution and samples of this solution were injected on an HPLC system with a cation exchange column directly after mixing during different time intervals. The increase of complex in the solution over time was fitted to a model describing association together with dissociation resulting in an association rate constant and a dissociation rate constant.

In the case of radiolabeled complexes, association of (dissociated) free radioactive metal ions with free ligand can be prevented by the addition of an excess of non radioactive metal ions to the complex solution (Jurkin et al., 2007; Denkova, 2006; van Doornmalen et al., 2002). The radioactive metal ions will be diluted by the non-radioactive metal ions which is called isotopic dilution.

Another method to prevent association of released metal ions with free ligand is by making the free metal ions unavailable for complexation which can be done by addition of cation exchange material to a dissociating complex (Wang et al., 1992) or by