Energy Transfer between Glucose and Dysprosium(III) in Aqueous Solution

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The fluorescence of Dy(III) and other lanthanides is enhanced when glucose is present in the solution and the solution has been heated (15 min at 175°C). The experimental evidence suggests that this is due to energy transfer between the aldehyde formed when glucose is heated and Dy. This effect was used for glucose determination in aqueous solution. A detection limit of 7 μM was obtained, with a linear response to 1 mM.

Keywords Energy transfer, enhanced fluorescence, glucose, lanthanide

Strong fluorescence, specific to the ion, may occur when the lanthanides are excited, as a consequence of non-radiative energy transfer from an excited species to the cation, followed by radiative emission from the latter. Intermolecular[1] and intramolecular[2] energy transfers have both been investigated and form the basis for many spectroscopic methods for the determination of lanthanides.[3] It is well known that some organic compounds, such as β-diketones form complexes with lanthanides and participate in an intramolecular energy transfer. The first work in this area was reported by Weissman[4] in the early 1940s.

Organic chelates of lanthanide ions, generally used to enhance the detection sensitivity of the ions, are also used in other applications, such as sensitive detection of the ligand, fluorescent labeling in biochemistry and clinical immnunology. Lanthanide chelates have also found use as shift reagents for NMR spectroscopy[5,6] and lanthanide ions have been used as luminescent chromophores for liquid chromatography.[7] For example, fluorescence enhancement of the ligand has been applied to the detection of tetracyclines.[8] Another example is the detection of theophylline (1,3-dimethylxanthine), alone[9] or in the presence of caffeine[10] by sensitized luminiscence of europium.

Recently, reducing sugars were shown to enhance the fluorescence of some lanthanides.[11] It was found that the native fluorescence of europium(III), terbium(III) and dysprosium(III) were markedly enhanced after heating with several reducing sugars.

In this work, we examine the mechanism for this enhancement and evaluate the procedure as a method for the determination of glucose.

Experimental

Reagents and chemicals

Dysprosium(III) oxide and gadolinium(III) oxide (SPEX, Industries Inc., Metuchen, NJ 08840 USA), europium chloride hexahydrate 99.9% (Alfa Products, 152 Andover St. Danvers, MA 01923, USA), terbium chloride hexahydrate 99.999% and DL-glyceraldehyde 95% (Aldrich Chem. Co., P. O. Box 355, Milwaukee, WI 53201, USA), acetaldehyde (Fisher Scientific), glutaraldehyde 25% in water (Eastman Kodak Company) and D-glucose anhydrous (Mallinckrodt Baker, Inc., Kentacky 40361, USA) were used. Solutions were prepared using purified water (Milli-Q, Millipore, Bedford, MA, USA).

Apparatus

All fluorescence measurements were obtained with a SPEX Fluorimeter (Fluorolog). The excitation and emission slits were both set at 2 mm (7.2 nm and 3.6 nm spectral bandpass, respectively). When dysprosium was used, the excitation wavelength was 283 nm, and the emission wavelength, 477 nm. A quartz cuvette with a 1-cm path length was used. A Hewlett Packard Model 8450A diode-array spectrophotometer was used for the absorption studies.

Procedure

Aqueous solutions containing about 50 mM dysprosium and glucose were prepared. Samples were placed in Pyrex test tubes with a Teflon screw cap (OD×L=16×125 mm) and heated at 175°C for 15 min.

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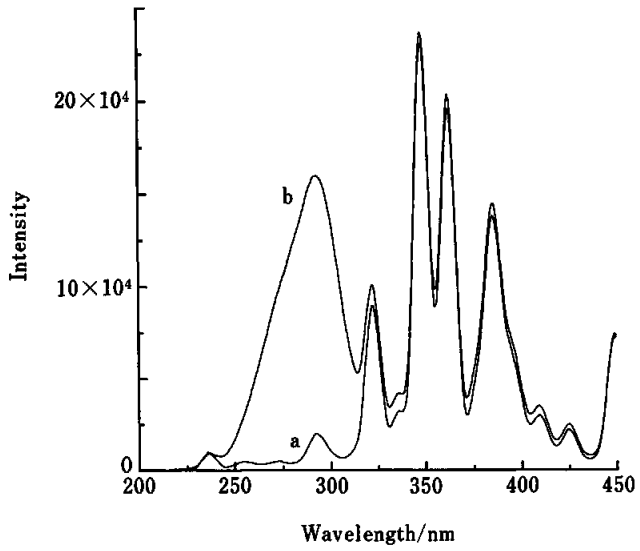

Fig. 1 Excitation spectra (λ_{em}=477 nm) of 50 mM dysprosium and 5 mM glucose, a) before and b) after heating.

A dry block bath was used for heating. The samples were then removed from the dry block and allowed to cool to room temperature. Fluorescence measurements were made using 283 nm as the excitation wavelength and 477 as the emission wavelength.

Results and Discussion

Modification of the excitation spectra

When a solution of glucose and dysprosium was heated, a large increase fluorescence signal in the excitation spectrum of dysprosium was observed (λ_{em}= 477 nm) in the region between 250 and 310 nm, as can be seen in Fig. 1. Therefore, 283 nm was chosen as the excitation wavelength because the largest increase was produced. Using other lanthanides, such as Eu and Tb, similar increases in the excitation spectra were also observed. For Gd, the effect was the opposite, *i.e.*, the signal decreased after the sample had been heated with glucose. This can be explained if the energy level structure of these elements is observed.[12] Figure 2 shows a schematic energy level diagram for each element and for the compound formed when a solution containing glucose and the lanthanide is heated. As can be seen, for Eu, Dy and Tb an energy transfer is possible from the compound formed to the lanthanide, however, for Gd, this is not possible because the lowest levels of the ion are located at energerically higher states than the compound. In the literature, a fluorescence signal at 310 nm was obtained for Gd.[13,14] However, we found experimentally a band at 620 nm, which can not be explained with the energy levels shown in Fig. 2.

Effect of temperature and heating time

First, a study of the influence of temperatures and times of heating was carried out. Temperature of 90, 150, 160 and 175°C and times of 15, 30 and 60 min were

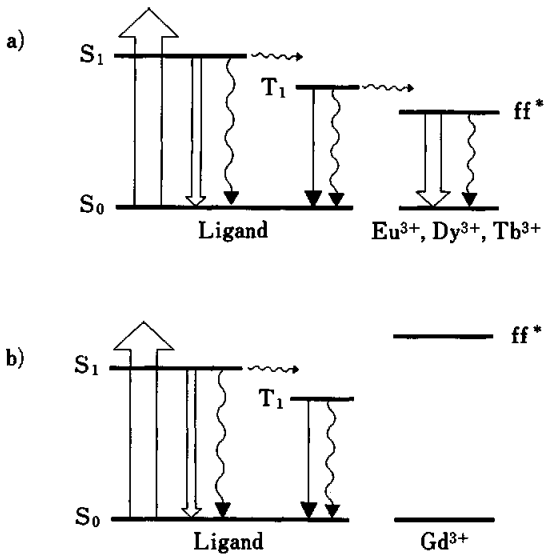

Fig. 2 Schematic energy level diagrams for the lanthanide-glucose complexes. The lowest ff* levels of the central metal ion are located at energetically lower (a) or higher (b) states than the lowest triplet level (T_1) of a ligand. Radiative, very weakly radiative, and nonradiative processes are represented by ⇒, →, and ⇝, respectively.

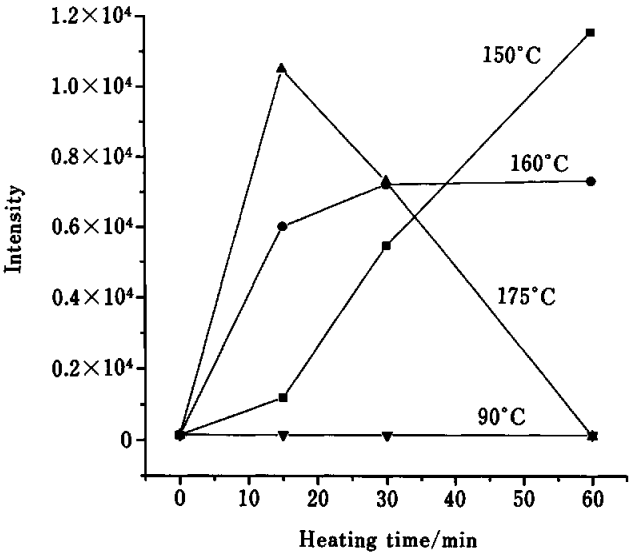

Fig. 3 Variation of fluorescence signal (λ_{ex}=283 nm, λ_{em}= 477 nm) of dysprosium and glucose solutions *vs.* heating time at 150, 160 and 175°C.

evaluated.

For all solutions a glucose concentration of 5 mM and a dysprosium concentration of 50 mM was used. The results are shown in Fig. 3, where the fluorescence signal at 477 nm is plotted *vs.* heating time, at 90, 150, 160 and 175°C. A temperature of 175°C and a time of 15 min were chosen as compromise experimental conditions, in order to avoid the inconveniently long 60 min heating time. Under these conditions, the fluorescence signal

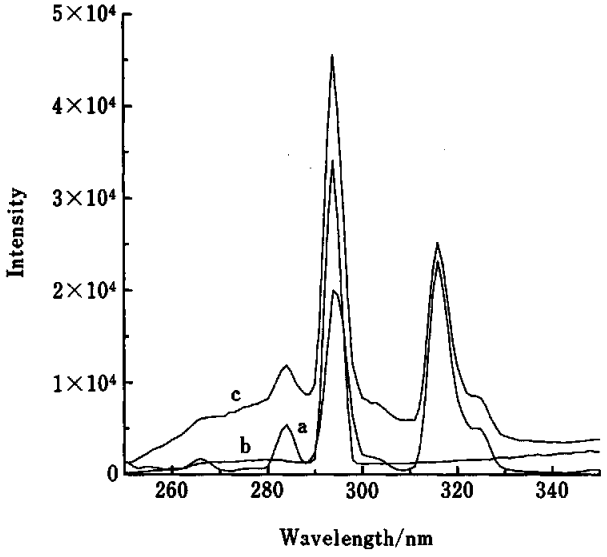

Fig. 4 Excitation spectra (λ_{em}=590 nm) of: a) 50 mM europium, b) 5 mM glutaraldehyde and c) 50 mM europium and 5 mM glutaraldehyde.

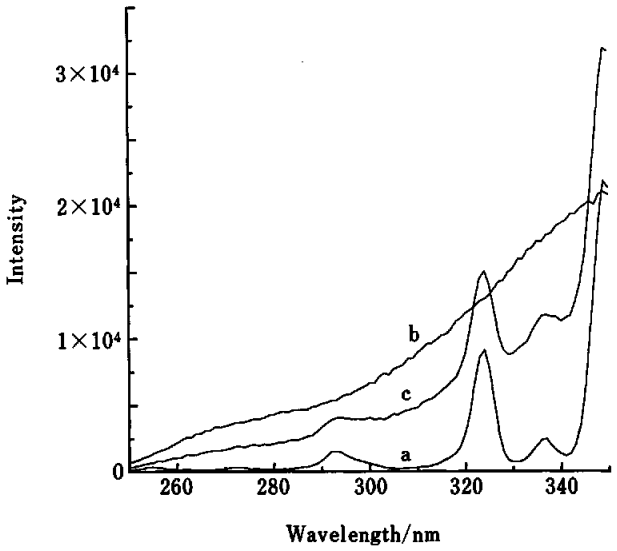

Fig. 5 Excitation spectra (λ_{em}=477 nm) of: a) 50 mM dysprosium, b) 5 mM glutaraldehyde and c) 50 mM dysprosium and 5 mM glutaraldehyde.

increased 70 times.

For Eu (λ_{ex}=250 nm and λ_{em}=590 nm), the best results resulted when the solution was heated at 160°C for 60 min, the signal increasing almost 9 times; and for Gd (λ_{ex}=271 nm and λ_{em}=620 nm) the signal disappeared completely when the same conditions as for Dy were used.

Energy transfer mechanism

Several studies were performaed in order to establish the mechanism of the process. First, molecular absorption spectra of the Dy-glucose solution was recorded before and after the heating step. New bands appeared after heating at 227 and 283 nm, with absorbance values of 0.139 and 0.514, respectively. When a solution of only glucose was heated, a much smaller increase in absorbance was observed (bands at 222 and 267 nm, with absorbance of 0.239 and 0.100, respectively). Apparently, when glucose was heated in the presence of Dy, a complex between Dy^{3+} and the opened ring glucose formed heating; the complex absorbed between 250 and 300 nm.

In order to see if the same effect was produced when both solutions, glucose and Dy, were heated separately, a solution of Dy and another of glucose were heated and mixed at room temperature. The resulting fluorescence spectra (λ_{ex}=283 nm) showed an increase in fluorescence intensity 8 times less when both solutions were heated separately compared to when they are heated together. It is reasonable to conclude that glucose forms an aldehyde when heated, and that the aldehyde group reacts with Dy. However, when the solution is allowed to cool to room temperature, the ring structure most likely formed again, because of an equilibrium at room temperature where the most stable form is a closed ring.

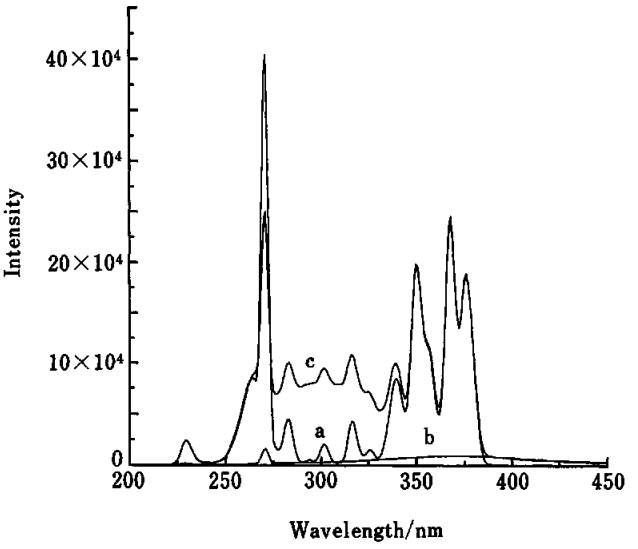

Fig. 6 Excitation spectra (λ_{em}=543 nm) of: a) 50 mM terbium, b) 5 mM glutaraldehyde and c) 50 mM terbium and 5 mM glutaraldehyde.

Because of this, the reaction of the aldehyde with Dy is much more efficient when they are heated together.

The solutions were also examined by other techniques, including Attenuated Total Reflectance IR and Raman Spectroscopy, but no definitive results were obtained.

If, as seemed likely, a carbonyl group was responsible for the process, a similar effect would be expected with other aldehydes. In order to confirm this, acetaldehyde, glutaraldehyde and glyceraldehyde were evaluated. Solutions containing these aldehydes and Dy were prepared and their fluorescence spectra were obtained at room temperature.

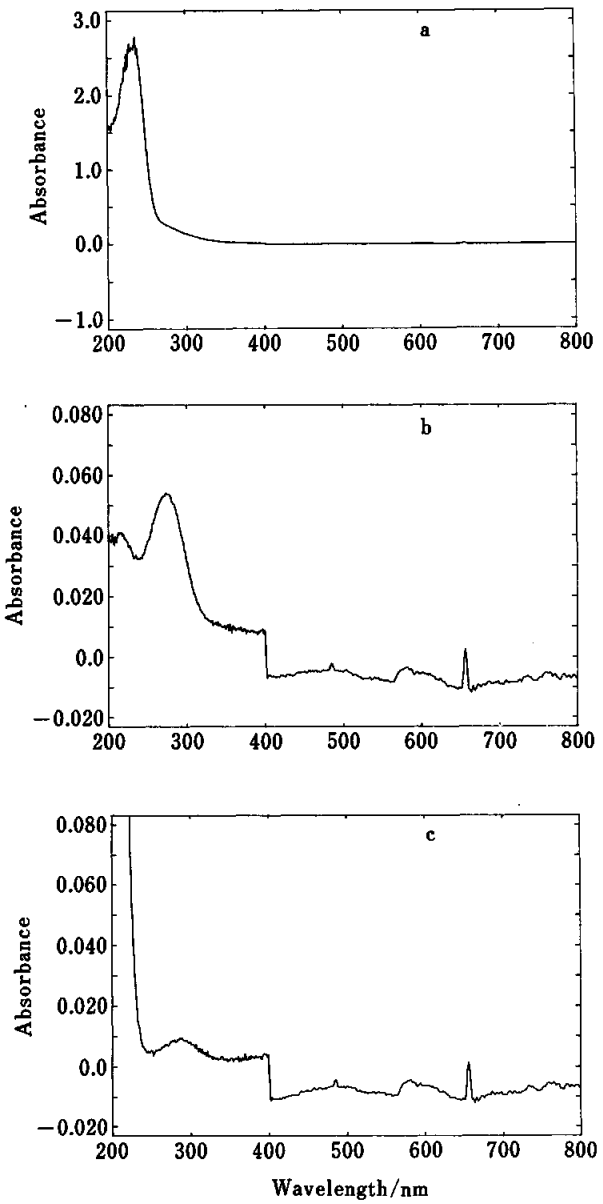

Fig. 7 Molecular absorption spectra of: a) 5 mM glutaraldehyde, b) 5 mM acetaldehyde and c) 5 mM gliceraldehyde.

Glutaraldehyde. Solutions containing 50 mM of Eu, Dy or Tb and 5 mM of glutaraldehyde were prepared. Figure 4 shows excitation spectra of Eu in the presence and absence of aldehyde. As can be seen, an increase in the fluorescence intensity of about 18 times was produced when glutaraldehyde was present. The largest enhancement was produced at 272 and 308 nm. However, when Dy was used, an increase in the signal was observed, but it was primarily because of the blank, as shown in Fig. 5. For Tb (Fig. 6), an increase in the intensity was produced between 275 and 330 nm.

Acetaldehyde. In this case, an increase in signal was only observed with Eu, and the change was much less than those seen for the heated glucose solutions.

Glyceraldehyde. No enhancement was observed using Eu, Dy or Tb.

Although the effect of the above aldehydes on the fluorescence of Eu, Dy, and Tb were not totally conclusive, the results seem to be generally in agreement with our proposed mechanism of energy transfer from the aldehyde to the rare earth ion. The absorption spectra of the three aldehydes (see Fig. 7) are in agreement with the fluorescence results, *i.e.*, the excitation is greatest for glutaraldehyde, much less for acetaldehyde, and nearly non-existent for glyceraldehyde.

Analytical characteristics

The enhancement effect was observed to be proportional to glucose concentration. Therefore, the enhanced fluorescence of trivalent dysprosium was utilized for the determination of glucose in aqueous solution and the main analytical characteristics were evaluated. An analytically useful range from 10 μM to 1 mM and a detection limit of 7 μM were obtained. The RSD was determined as follows: five different solutions containing 50 mM of Dy and 0.1 mM of glucose were prepared, heated and measured three times each. An RSD of 10% was obtained, however if it was calculated for each individual solution, an average of 3.5% was obtained. This shows the importance in controlling the heating process.

S. Cabredo thanks the University of La Rioja for a leave and for financial support.

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(Received August 19, 1996)
(Accepted December 20, 1996)