β-Cyclodextrin - Enhanced Fluorimetry of Polyaromatic Dialdehyde - Derivatives and its Application to Amines and Amino Acids Determination

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Summary: An investigation of naphthalene-2,3-dicarboxaldehyde (NDA) as a potential derivatising reagent for the fluorimetric determination of amines and amino acids in the presence of β-cyclodextrin has been conducted. Enhanced fluorescence was observed in most cases. Experimental conditions, including pH of the medium, are optimised for the formation of the fluorescent NDA-derivatives. The fluorimetric determination of amines and amino acids by the β-cyclodextrin - enhanced fluorimetry is reported alongwith an expected possible mechanism of these β-cyclodextrin - included reactions.

Introduction

The fluorescence exhibited by excited organic molecules in solution depends mainly on the existence of extended π conjugation bonds. These molecules which are simple such as amines and amino acids (glycine, arginine, taurine etc.) do not show any fluorescence unless derivatised with a suitable fluorigenic reagent. The commercially available reagents include *o*-phthalaldehyde [1], fluorescamine [2], dansyl chloride [3], 7-chloro-4-nitrobenzo-2-oxa-1,3-diazole (NBD-Cl) [4] etc. The OPA derivatives are fluorescent but suffer from spectral interferences for study of biological fluids due to fluorescence emission taking place at shorter wave-length [450 nm]. The present study is aimed to replace the old fluorigenic reagent [1] with a laboratory synthesized fluorigenic reagent [5] called naphthalene-2,3-dicarboxaldehyde. This reagent like OPA reacts with compounds containing -NH_2 group in the presence of a thiol in alkaline medium to produce water soluble isoindole, a fluorescent product [1]. The fluorescence emission, however, unlike OPA-derivatives takes place at longer wave-length and thus the fluorescence measurement is considered as interference free for studies of the biological

samples. This is thus an advantage in the use of this reagent. However, it has been observed that intensity of fluorescence is not appreciable without the addition of β-cyclodextrin (βCD). Thus the present investigation is intended to have the above derivatisation reaction coupled with the inclusion of β-cyclodextrin (a major product from the reaction between the enzyme cyclodextrin transglycosylase and a starch solution pretreated with α-amylase) for enhanced fluorimetry. It is expected that the inclusion of βCD would provide enhanced absorption leading to an enhanced fluorescence emission.

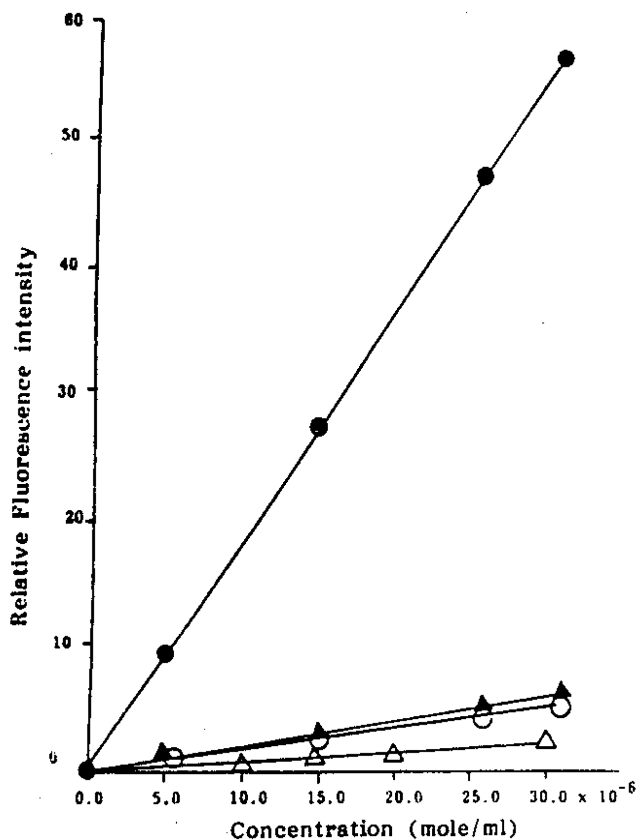
Results and Discussion

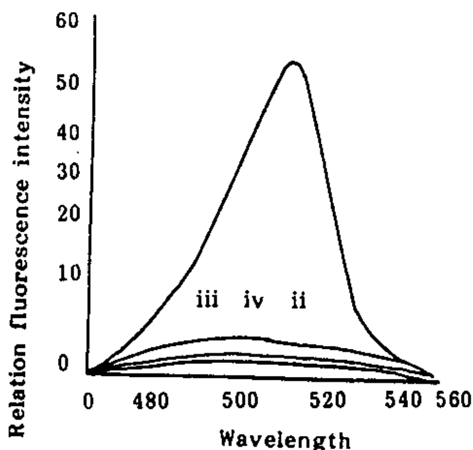
Reaction using β-cyclodextrin as fluorescence enhancer

Naphthalene-2,3-dicarboxaldehyde (NDA) formed a fluorescent derivative with amino acids but the intensity of fluorescence emission was very low. A preliminary investigation confirmed that like OPA-derivatisation reaction [1], this reaction (with NDA) also needs to be carried out in alkaline medium (pH 10.0). It was observed that fulfillment of this condition alone did not give satisfactory results. The intensity of fluorescence, shown in Fig. 1, is seen to be smallest over a varying concentration of glycine in absence of β-cyclodextrin. Of the three cyclodextrins (α, β and γ), βCD was found to enhance the fluoresence intensity of the NDA-derivative by nearly 8 times, Fig. 1 (a,b) compared with the NDA-derivative without βCD. A quality of 2.5 mmol/l of βCD was sufficient to give maximum enhancement of fluorescence. The other two dextrins proved to be of very little importance with respect to fluorescence enhancement.

Mechanism of fluorescence enhancement by βCD

The fluorescence of the NDA glycine derivative is considerably enhanced by the addition of βCD in the solution of derivatisation reaction. It seems that in the situation the complex finds a new microenvironment in which hydrophobic and electrostatic forces acting simultaneously are able to





Emission spectra of NDA-glycine derivative (Glycine 30.0 x 10^{-3}M) Cyclodextrins: (i) (ii) (iii) (iv) absent.

● NDA-Glycine + BCD

▲ NDA-Glycine · CD

○ NDA-Glycine · CD

△ NDA-Glycine

Fig. 1: Effect of cyclodextrins (α, β and γ) on fluorescence intensity.

shelter the singlet state. The formation of inclusion complexes is made possible by the architecture of the cyclodextrin molecule. In βCD there are seven glycopyranose units and are connected via the 1 and 4 carbon atom (Fig. 2 (a)). All the glucosyl-*O*-bridges point into the centre of the molecule, producing a torus shape (barrel), Fig. 2(b). The primary hydroxyl groups project from one outer edge of the barrel, and the secondary hydroxyl groups from the other. The result is a molecule containing cavity with a hydrophobic centre because of high electron density of the glucosidic oxygen and a relatively hydrophobic outer surface. The hydrated βCD molecule i.e. with water molecules in the cavity represents a high energy state which readily accepts a guest molecule in place of the water. The more hydrophobic or insoluble the guest molecule, the more readily it will form a complex, thus reducing the chance to

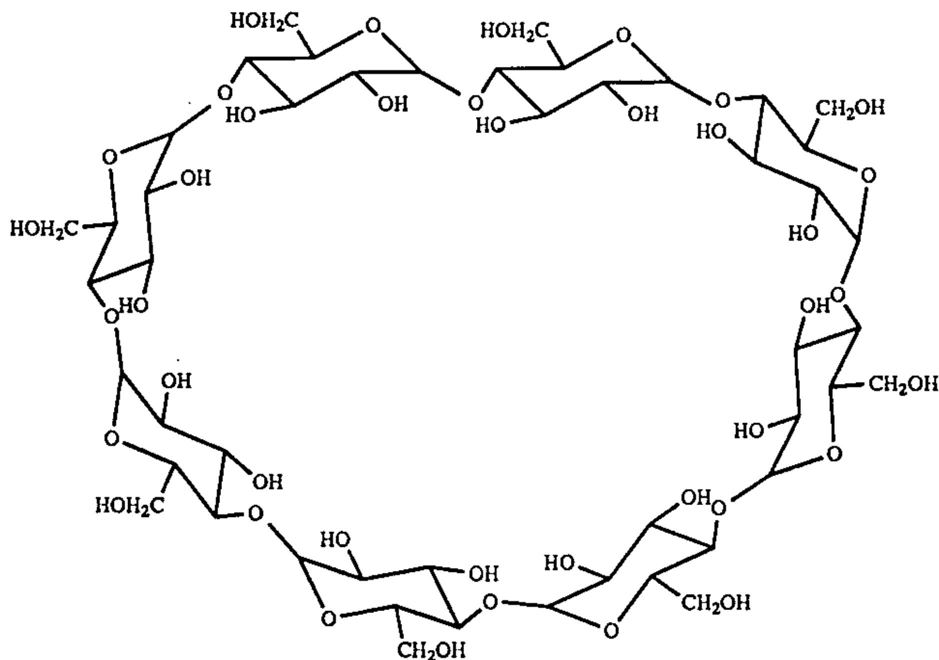


Fig. 2 (a) β-Cyclodextrin

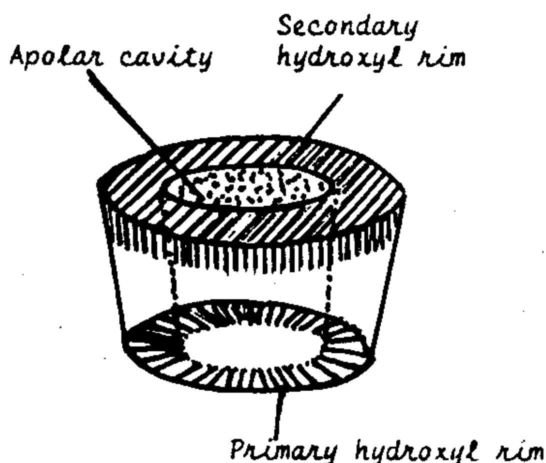


Fig. 2b:The molecular shape of βCD showing the central cavity.

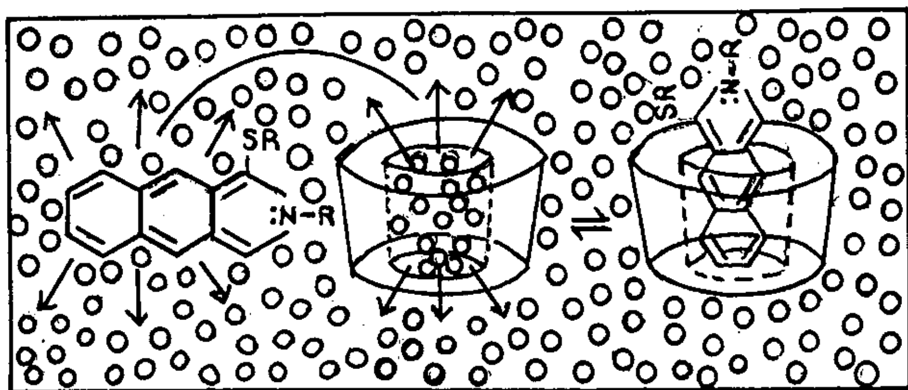


Fig. 3: The formation of βCD complexes in aqueous solution.

dissociate. The increase in the fluorescence of the NDA-glycine derivatives in Fig. 1 may be due to the formation of an inclusion complex [6], Fig. 3, where the hydrophobic molecule (NDA-derivative) may have been transported into the βCD hydrophobic interior of 7.5 Å internal diameter to include the whole of the isoindole molecule, thus giving increased fluorescence. Reactions that are done in presence of βCD can also be stereoselective [7].

Emission and excitation spectra of NDA-glycine derivatives

The emission and excitation spectra (uncorrected) of NDA-glycine and the blank in water in the presence of β-cyclodextrin (βCD) is shown in Fig. 4. The excitation maxima at 462 nm is highly intense. By exciting the derivatives at this wavelength an emission peak at 520 nm is produced: the fluorescence is maximum for derivatives formed at pH 10.0.

The blank is run under identical reaction conditions utilizing the same reagents and solvent (water) as the amino acids, except the amino acid solution is replaced by an identical solvent volume (water). To minimize errors, it is recommended that a blank be run parallel to amino acid determination and its fluorescence intensity be subtracted from that of the amino acids.

Fluorescence intensity, concentration and sensitivity

The relationship of the fluorescence intensity of the NDA-glycine to glycine concentration was studied using glycine as a generic model. Fig. 1 is a plot of the fluorescence intensity of the NDA-derivative versus concentration of glycine. The fluorescence intensity is linear with glycine concentration upto a concentration of 30.0 x 10^{-6} mole/ml (2.67 mg/ml); the correlation coefficient was calculated to be 0.995.

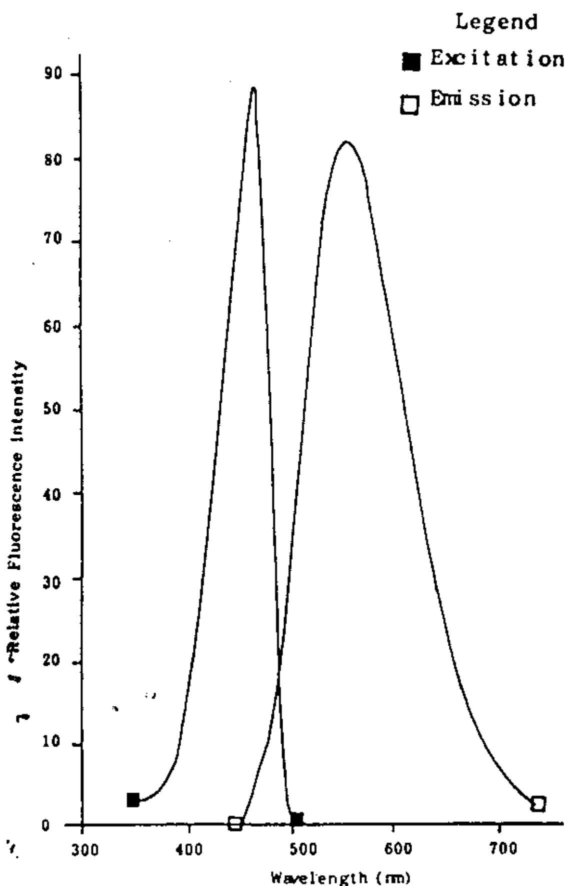


Fig.4: Excitation and emission spectra of NDA-glycine derivative obtained with ethane-thiol.

The limit of detection was estimated to be 0.63 nmole/ml (4.72×10^{-8} g/ml).

Experimental

Apparatus

Fluorescence measurements were made with an MPF-44B spectrofluorimeter (Perkin-Elmer). The desired pH value of a solution was adjusted by the use of a Corring digital pH meter model PTI-S.

Solution

(i) A measured quality of ortho-phthalaldehyde (OPA) or naphthalene-2,3-dicarboxaldehyde (NDA) was dissolved in a few ml of ethanol and made upto the required volume with distilled water to obtain a solution of known molarity.

(ii) A 10.9 mM β-cyclodextrin was prepared by dissolving its required quantity in appropriate volume of distilled water using ultra sound.

(iii) Borate buffer of pH 10 was prepared from borax (4.8 g) by dissolving in distilled water and making up the volume to one litre.

(iv) A 0.1% v/v of different thiol solutions were prepared using either ethanol or distilled water.

All other reagents were of analytical grade.

Fluorescent labelling (Derivatisation Reaction)

Procedure

0.2-0.3 ml of very dilute solution (10^{-4}M) of the sample (amine and amino acids) was mixed with 1 ml of 10.9 mM β-cyclodextrin, followed by the addition of 0.6 ml of borate buffer of pH 10. 0.1 ml of 0.1% v/v aqueous thiol solution and finally the solution of a suitable fluorigenic reagent in slightly excess over the sample. The volume was made upto 5 ml with water and the fluorescence emission was measured at a suitable wavelength (520 mm for NDA-derivatives).

Reagents blanks lacking amines or amino acids were prepared and used as a reference.

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