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Photoinduced electron transfer between cytochrome *c* and a novel 1,4,5,8-naphthalenetetracarboxylic diimide with amphiphilic character

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Abstract

N-dodecyl-*N′*-(2-phosphonoethyl)-1,4,5,8-naphthalenetetracarboxylic diimide (DNDI) is a novel naphthalenic diimide with amphiphilic character. DNDI was synthesized through the sequential reaction of 1,4,5,8-naphthalenetetracarboxylic dianhydride, first with dodecylamine and then with 2-aminoethylphosphonic acid. Fluorescence measurements showed that DNDI forms excimers in water at sufficiently high concentrations. The fluorescence quantum yield of DNDI in diluted solutions is sensitive to the polarity of the microenvironment, decreasing as going from water to less polar solvents. This property allowed to monitor the incorporation of DNDI into cetyl trimethyl ammonium bromide (CTAB) micelles, with a binding constant of $1.2 \times 10^4\ \mathrm{M}^{-1}$. UV irradiation (365 nm) of solutions containing DNDI and the redox protein cytochrome *c* (cyt *c*) resulted in the reduction of the heme iron from the Fe(III) to the Fe(II) state, a reaction that was inhibited by the incorporation of DNDI into CTAB micelles. DNDI formed host–guest complexes with α-cyclodextrin (α-CD) through the inclusion of the dodecyl group, resulting in an increased aqueous solubility of the compound.

Keywords: 1,4,5,8-naphthalenetetracarboxylic diimides; Micelles; Cytochrome *c*; Photoinduced electron transfer; Cyclodextrins

1. Introduction

1,4,5,8-Naphthalenetetracarboxylic diimides (NDI) are stable aromatic molecules with very interesting photophysical and photochemical properties [1–5]. They are easily reduced, giving rise to stable imide radical anions [1–3,6,7] that can interact with biological molecules. The biological activity of NDI was first studied by Yen et al. [8], who found out that some NDI derivatives form stable intercalation complexes with DNA. Irradiation of these complexes leads to the photoinduced cleavage of the DNA strands [9]. According to recent studies, photochemical damage to the DNA involves an electron transfer reaction from a guanine residue to the triplet excited state of NDI, generating a NDI radical anion together with oxidized guanine, resulting in DNA cleavage [10]. The imide radical generated in this way can react further with the DNA, causing both oxygen-dependent (type II) and oxygen-independent (type I) biological damage [3]. These studies have shown that NDI are potential candidates in the photodynamic therapy for cancer.

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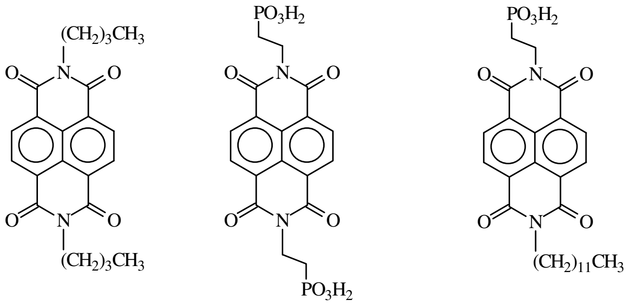
The NDI are easily synthesized through the reaction between 1,4,5,8-naphthalenetetracarboxylic dianhydride and primary amines. This reaction is very versatile, allowing the incorporation of virtually any N-substituent to the diimide, as long as a primary amine with the required substituent attached to the amino group is available. Previous studies reported by this group have described the synthesis and characterization of NDI derivatives containing either hydrophobic substituents (*N*,*N*′-bis(butyl)-1,4,5,8-naphthalenetetracarboxylic diimide) (BNDI) [4] or hydrophilic substituents (*N*,*N*′-bis(2-phosphonoethyl)-1,4,5,8-naphthalenetetracarboxylic diimide) (PNDI) [5] (Chart 1). BNDI is completely insoluble in water, but dissolves well in organic solvents, such as chloroform or acetonitrile. PNDI, on the other hand, is moderately soluble in water, but is insoluble in low polarity solvents. These two compounds allowed us to investigate the properties of NDI over a wide range of solvent polarity [4,5].

In the present paper, we describe the synthesis and physicochemical characterization of *N*-dodecyl-*N*′-(2-phosphonoethyl)-1,4,5,8-naphthalenetetracarboxylic diimide (DNDI), a novel NDI with amphiphilic character (Chart 1) that incorporates features of both BNDI and PNDI. This compound was chosen because it has a great potential for incorporation in new materials with biological applications. Its amphiphilic structure, with a polar head and an apolar tail, makes DNDI a strong candidate for incorporation into micelles. The presence of the phosphonate group, on the other hand, should allow the incorporation of DNDI into materials based on zirconium phosphonate chemistry. Such materials have biological applications in the photodynamic therapy for cancer [3,10] and as model systems for photosynthesis [2,11]. Furthermore, NDI-containing materials can be used as field-effect transistors [12], organic conducting polymers [6] and laser dyes [7], among other applications.

The most interesting property found here for DNDI, however, was the occurrence of a photochemical reaction with the redox protein cytochrome *c*, resulting in the reduction of the heme iron atom. This reaction has potential applications in photosynthetical devices, in biosensors and others.



BNDI PNDI DNDI

Chart 1. Structure of 1,4,5,8-naphthalenetetracarboxylic diimides containing N-substituents of varying polarity.

2. Experimental section

2.1. Materials

The following reagents were purchased from Aldrich Chemical Co. (Milwalkee, WI, USA): 1,4,5,8-naphthalenetetracarboxylic dianhydride, *N*-dodecylamine, 2-aminoethylphosphonic acid, imidazole, α-cyclodextrin (α-CD) and 2-hydroxypropyl-α-cyclodextrin (HP-α-CD). Cetyl trimethyl ammonium bromide (CTAB), sodium dodecyl sulfate (SDS), and horse heart cytochrome *c* (cyt *c*) were purchased from Sigma Chemical Co (St Louis, MO, USA). CTAB was recrystallized from acetone/methanol prior use. All the other reagents were used as received. All solvents and inorganic salts employed were analytical grade. Aqueous solutions were prepared with deionized water (Barnstead easypure RF system, Dubuque, IA, USA).

2.2. Instruments

UV–visible absorption spectra were recorded in a Shimadzu Multi-Spec 1501 spectrophotometer (Tokyo, Japan). Fluorescence measurements were taken with a Hitachi F 2500 Fluorescence Spectrophotometer (Tokyo, Japan). Irradiation of the samples was performed at 365 nm, with a 4 W compact lamp, model UVGL-25 (UVP, Upland, CA, USA). ^{1}H NMR spectra and elemental analysis (C, H, N) were done by the Analytical Center at the Chemistry Institute, Universidade de São Paulo (São Paulo, Brazil).

2.3. Methods

2.3.1. Synthesis of DNDI (Scheme 1)

(a) *N*-dodecyl-1,4,5,8-naphthalenetetracarboxylic monoimide: A suspension of 1,4,5,8-naphthalenetetracarboxylic dianhydride (4.26 g, 15.9 mmol) in *N*,*N*-dimethylformamide (DMF) (20 mL) was heated at 120–140 °C. A solution of *N*-dodecylamine (0.589 g, 3.18 mmol) in DMF (10 mL) was added dropwise within 1 h. The mixture was heated for another hour, then cooled down and poured into 300 mL of cold ethyl ether, resulting in the precipitation of a slightly brown solid. The solid was filtered off, washed with ethyl ether and dried under vacuum. The monoimide was extracted from the solid mixture, which contained