Journal of Photochemistry and Photobiology A: Chemistry 208 (2009) 50–58

Contents lists available at ScienceDirect

Journal of Photochemistry and Photobiology A: Chemistry

journal homepage: www.elsevier.com/locate/jphotochem

On the mechanism of the photoinduced reduction of an adduct of ferricytochrome C with a poly(4-vinylpyridine) polymer containing –$Re^{I}(CO)_3$(3,4,7,8-tetramethyl-1,10-phenanthroline) pendants

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ARTICLE INFO

Article history:
Received 2 July 2009
Received in revised form 6 August 2009
Accepted 14 August 2009
Available online 20 August 2009

Keywords:
Cytochrome c
Rhenium
Polymers
Photoinduced
MLCT
TEA

ABSTRACT

Changes in the UV–vis absorption spectrum revealed the formation of adducts between the Re^{I} polymer and ferricytochrome C, Fe^{III}-Cyt c. Different morphologies for the Re^{I} polymer and the adducts formed between the Re^{I} polymer and Fe^{III}-Cyt c were observed by TEM. The reduction of the Re^{I} chromophores in the polymer, achieved by the reductive quenching of the MLCT excited state of the Re^{I} polymer by triethylamine (TEA) and/or by the reaction between e^{-}_{solv} and $\{[(vpy)_2vpyRe^{I}(CO)_3(tmphen)^{+}]\}_{n\sim200}$ in pulse radiolysis experiments, produces $-Re^{I}(CO)_3(tmphen)^{\bullet}$ and $-Re^{I}(CO)_3(tmphenH)^{\bullet+}$ as the main species. The reductive quenching of the MLCT of the Re^{I} polymer by TEA was followed by a rapid electron transfer from the $-Re^{I}(CO)_3(tmphen)^{\bullet}$ to the Fe^{III} center in the heme to produce ferrocytochrome C, Fe^{II}-Cyt c.

1. Introduction

Fe^{III}-Cyt c is a small protein, ~12 kDa, consisting of a single 104 amino acid peptide with a single heme group, Scheme 1. Because of its ubiquitous nature and sequence homology, Fe^{III}-Cyt c has been used as a model protein for molecular evolution [1–3,4a]. Fe^{III}-Cyt c is primarily known as an electron-carrying mitochondrial protein. The interconversion of Fe^{II}-Cyt c and Fe^{III}-Cyt c within the cell makes it an efficient biological electron carrier and it plays a vital role in cellular oxidations in both plants and animals. It is generally regarded as a universal catalyst for the respiratory process, forming an essential electron bridge between the substrates and oxygen. At a cellular level, its main function is to transport electrons from cytochrome c reductase to cytochrome c oxidase. Biological synthesis of Fe^{III}-Cyt c occurs in the intermembrane space of mitochondria. It involves heme ligation to apo Cyt c, which has a disordered structure in solution, and subsequent formation of the folded (native) Fe^{III}-Cyt c. On the other hand, the interaction of folded Fe^{III}-Cyt c with lipids resulted in a partial unfold of the native protein, and Fe^{III}-Cyt c was shown to exist in equilibrium between a soluble state and a membrane-bound state at physiological pH [4b]. Moreover, proteins that are partially or wholly disordered under physiological conditions can still perform important biological functions, such as molecular recognition, signaling, and regulation [4c]. Because the biological properties of proteins arise mainly from their native conformations, during the past 70 years considerably more attention has been focused on the native conformations than on the nonnative ones. In the past few years, however, there has been increasing interest in nonnative and denatured states of proteins since these less ordered states play important roles in at least three major phenomena: (1) Protein folding and stability, (2) Transport across membranes and (3) Proteolysis and protein turnover [4d,4e].

The goal of developing a detailed understanding of inter-protein electron transfer involves many complex problems since the reactions are dependent on many different factors such us electronic properties of the redox centers, the distance and pathway of electron transfer, the reorganization energy and the kinetics of complex formation and dissociation. The measurement of the rate of intramolecular electron transfer has been a particularly difficult problem since only a limited number of techniques are available which include stopped-flow spectroscopy, pulse radiolysis, flash photolysis [5a] and lately spectrolelectrochemistry combined with surface enhanced resonance Raman spectroscopy [5b].

Intramolecular electron transfer betweeen Fe^{III}-Cyt c and transition metal compounds has been investigated by attaching photoactive Ru complexes to the protein surface [5a,6–8]. In those studies, photoinduced electron transfer occurs between the

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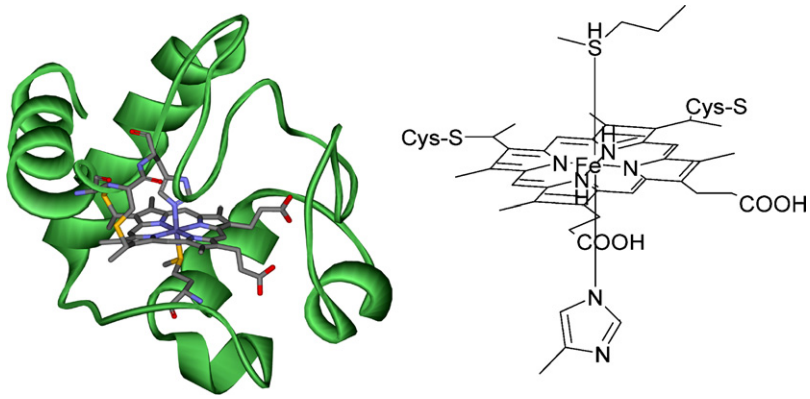
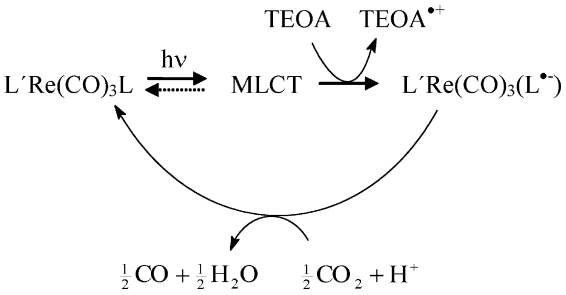
doi:10.1016/j.jphotochem.2009.08.003



Scheme 1.

reducing excited state of $Ru^{II}(bpy)_2B_L^{2+}$ (B_L = bridging ligand) and the ferric heme group. However, as far as we know, there are no photoinduced electron transfer studies between Fe^{III}-Cyt c and Re^{I} complexes. $Re^{I}(CO)_3LL'$(L = diimine, L′ = halide or pyridine derivative) complexes are capable of acting as photocatalysts with the aid of a sacrificial reductant, eq. (1),



(L = polypyridine or phenanthroline derivative) (1)

It is known that reductive quenching of the lowest Re-to-bpy charge transfer excited state (MLCT) of the complex by triethanolamine (TEOA) generates a species, $[ClRe(CO)_3(bpy)]^{\bullet -}$, capable, for instance, of mediating the two-electron process of CO_2 reduction to CO [9].

On the other hand, numerous studies have dealt with thermal and photochemical reactions of inorganic polymers in the solid state and solution phase. Interest in their photochemical and photophysical properties is driven by their potential applications in catalysis and optical devices [10–21]. The photophysical properties in the solution phase of a Re^{I} containing polymer $[(vpy)_2vpyRe(CO)_3(tmphen)^+]\}_{n\sim200}$ (where tmphen = 3,4,7,8-tetramethyl-1,10 phenanthroline and vpy = 4-vinylpyridine, see Scheme 2), hereafter designated as ReP4VP, were investigated in previous work [22]. As poly(4-vinylpyridine) tends to interact with Fe^{III}-Cyt c by hydrogen bonding interactions [1], we decided to study the photocatalytic properties of the chromophores $Re(CO)_3(tmphen)^+$ in the reduction of Fe^{III}-Cyt c by using the polymer ReP4VP as a probe, since ReP4VP also interacts with Fe^{III}-Cyt c by the formation of adducts. In this paper we explore the photoinduced reduction of Fe^{III}-Cyt c by ReP4VP in CH_3CN/H_2O solutions, where the protein is denatured. The reductive quenching of the MLCT excited state of ReP4VP by triethylamine (TEA) produces -$Re^{I}(CO)_3(tmphen)^{\bullet}$ species in the polymer, which in the presence of Fe^{III}-Cyt c reduce the ferric iron of the heme portion of the protein to the ferrous state.

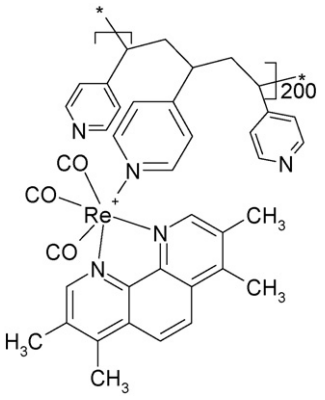
2. Materials and methods

2.1. Flash-photochemical procedures

Optical density changes occurring on a time scale longer than 10 ns were investigated with a flash photolysis apparatus described elsewhere [23–25]. In these experiments, 25 ns flashes of 351 nm (ca. 25 - 30 mJ/pulse) light were generated with a Lambda Physik SLL-200 excimer laser. The energy of the laser flash was attenuated to values equal to or lower than 20 mJ/pulse by absorbing some of the laser light by $Ni(ClO_4)_2$ solutions with appropriate optical transmittances, $T = I_t/I_0$, where I_0 and I_t are the intensities of the light arriving at and transmitted from the photolysis cell, respectively. The transmittance, $T = 10^{-A}$, was routinely calculated by using the spectrophotometrically measured absorbance, A, of the solution. A right angle configuration was used for the pump and the probe beams. Concentrations of the complexes were adjusted to provide homogeneous profiles of photogenerated intermediates over the probe beam optical path, l = 1 cm. To satisfy this optical condition, solutions were prepared with an absorbance equal to or less than 0.4 over the 0.2 cm optical path of the pump. All solutions used in the photochemical work were deaerated with streams of ultrahigh-purity N_2 before and during the irradiations.

2.2. Pulse radiolysis

Pulse radiolysis experiments were carried out with a model TB-8/16-1S electron linear accelerator. The instrument and computerized data collection for time-resolved UV–vis spectroscopy and reaction kinetics have been described elsewhere in the literature [26,27]. Thiocyanate dosimetry was carried out at the



Scheme 2.