Coordination Chemistry Reviews 255 (2011) 737–756

Contents lists available at ScienceDirect

Coordination Chemistry Reviews

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

Review

Photochemical reagents for the study of metalloproteins by flash photolysis

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Contents

ARTICLE INFO

Article history:
Received 28 September 2010
Accepted 17 November 2010
Available online 25 November 2010

This article is dedicated to Harry B. Gray, a great practitioner and ambassador of chemistry, on the occasion of his 75th birthday.

Keywords:
Flash photolysis
Metalloproteins
Caged reagents
pH-jump

ABSTRACT

Flash photolysis has been used extensively in the advancement of our understanding of the electron transfer reactivity of metalloproteins and in investigation of the kinetic complexities of electron transfer-initiated protein folding. Additional opportunities for the use of flash photolysis to understand the functional properties of metalloproteins have been afforded through the use of photoactive caged complexes. This review surveys the uses of caged complexes to the study of metalloproteins that have been reported and considers the potential for expanded use of photoactive caged complexes that have not yet been applied in this manner.

1. Introduction

Following the initial reports of Norrish and Porter [1–3] that a brief, highly intense light burst can be used to initiate photochemical reactions and enable the spectroscopic detection of transient chemical species, this method was soon applied to the investigation of biological processes. Early biological applications of flash photolysis concerned the visual pigment rhodopsin [4], the photosynthetic pigment chlorophyll [5,6], and rebinding of carbon monoxide to hemoglobin [7,8]. Subsequently, DeVault and Chance used flash photolysis of photosynthetic proteins at cryogenic temperature to provide the first experimental evidence for electron tunneling in a biological electron transfer reaction [9,10]. Together, these pioneering studies demonstrated the value of flash photoly-

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doi:10.1016/j.ccr.2010.11.025

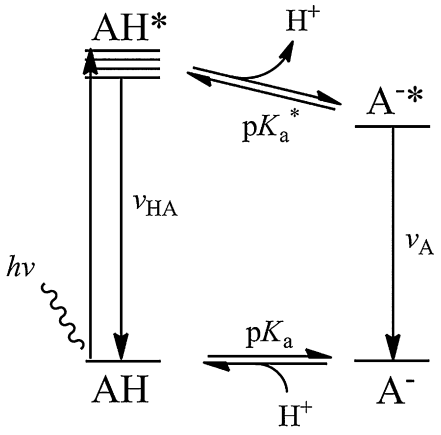
sis spectroscopy as a means of characterizing the kinetics of rapid chemical processes in biological systems and led to development of increasingly sophisticated instrumentation and chemical strategies for use in biological studies. These improvements in technologies in turn resulted in the application of flash photolysis to investigation of a broader range of biological phenomena.

While these pioneering studies focused on chemical systems that are inherently photosensitive, the use of small, photoactive molecules to initiate chemical reactions involving photolytically unreactive biological systems made flash photolysis far more generally applicable. Thus, flavins [11,12], inorganic coordination compounds [13], and reduced pyridine nucleotides (NADH) [14] were useful reagents for the study of metalloprotein electron transfer kinetics and have been used by a number of investigators to characterize an increasing number of biological systems. The use of photoactive reagents in this way has been refined subsequently by Gray and colleagues through the design and synthesis of enzyme substrate analogues with appended photoactive functional groups. These molecular "wires" enable photo-initiated electron transfer to structurally inaccessible active sites that otherwise exhibit little or no photochemical response to exogenous reagents [15–17].

Another category of small, photoactive reagents that has been developed over the past 30 years for use in flash photolysis experiments is comprised of compounds that undergo rapid photolytic release of a chemical species to initiate a chemical reaction. Following the terminology introduced by Kaplan et al. [18], reagents of this type are generally referred to as "caged" compounds, a term that has had a variety of alternative chemical meanings in other contexts. The first reports of caged compounds of this type concerned caged cAMP [19] and caged ATP [18]. Since then, a great number of photoactive caged complexes has been reported that permit the rapid but controlled release of a wide range of biologically active chemical species that vary in complexity from protons (vide infra) to proteins [20–24]. The current review surveys those caged compounds that have been used in or may have the potential for use in mechanistic studies of metalloproteins in aqueous solution. From this survey, it should be apparent that although the value of this experimental approach has been well established, many new and informative opportunities remain to be developed through application of this technology.

2. pH-jump reagents

Among the earliest type of flash photolysis reagents to be reported are those capable of inducing rapid changes in solution pH. This early start can be credited in part to Weber, who in 1931 observed that the absorption and fluorescence emission spectra of 1-naphthylamine-4-sulfonate (**1a**) exhibit different dependences on pH [25]. This report sparked considerable activity concerning investigation of the acid–base equilibria of molecules in the excited



$$pK = pK_a - pK_a^* = \frac{0.625}{T} \times (\bar{\nu}_{HA} - \bar{\nu}_A)$$

Fig. 1. Simplified Jablonski diagram illustrating a thermodynamic cycle for excited-state proton transfer.

state (Fig. 1). One consequence of this work and related studies concerning excited-state proton transfer by Förster [26] and Weller [27] among others was recognition by the groups of Winn [28], Gutman [29] and Irie [30] that this phenomenon can be used as a means to perturb chemical and biological systems.

In principle, any molecule that exhibits a pK_a shift upon electronic excitation could be used for pH-jump studies, but the suitability of a reagent for this purpose depends on many factors [31,32]. As with all reagents reviewed here (Plate 1), pH-jump reagents must be sufficiently soluble in water. The magnitude of photo-initiated pH change will depend on the photochemical quantum yield (Φ_{pc}), the concentration of reagent and the change in pK_a between the ground and the excited states. These reagents should also be chemically inert with respect to the analyte. Reactions between analyte and reagent should be limited to proton transfer reactions unless, as in the studies by Gutman et al. [33–36], the experimental results are derived from absorbance and fluorescence changes of the reagents that are modulated by their non-covalent interactions with the analyte. Next, reagents should exhibit either mildly alkaline or acidic pK_as in the ground-state and a substantial pK_a shift upon excitation ($\Delta pK_a \sim 3$–7 pH units; Table 1) so that proton transfer reactions are energetically favourable. The compilation by Ireland and Wyatt [37] of ground- and excited-state pK_a values of a wide range of organic compounds which exhibit photo-induced pK_a shifts is a valuable source of such information. This database has been augmented over the years [38–40] and, more recently, it has been expanded by Vos to include a number of $[Ru(bipy)_2L]^{2+}$ complexes [41]. In cases where these data are unavailable for a

Table 1
Kinetic and thermodynamic parameters of reagents used in pH- and pOH-jump experiments.

Compound	Jump[a]	λ_{ex} (nm)	pK_a	pK_a^*	k_{act}[b] ($\times 10^9$ s^{-1})	k_{recomb} ($\times 10^9$ M^{-1} s^{-1})	Refs.
Pyranine (8-hydroxypyrene-1,3,6-trisulfonate)	pH-	347.2	7.7	0.5	10	160	[33]
				1.4	32		[252]
							[253]
2-Naphthol-3,6-disulfonate	pH-	347.2		0.5	31	70	[253]
							[254]
2-Naphthol-6-sulfonate	pH-	347.2	9.1	1.6	1.02	90	[37]
							[28]
Triphenylmethane leucohydroxide	pOH-						
6-Methoxy-quinoline	pOH		5.18	5.2			[37]
Acridine	pOH		5.5	10.6			[37]

[a] pH- and pOH- denote, respectively, the photoacidic and photobasic character of the reagent.
[b] k_{act} is the rate constant for proton dissociation of photoacids or proton abstraction for photobases.