Coordination Chemistry Reviews xxx (2017) xxx–xxx

Contents lists available at ScienceDirect

Coordination Chemistry Reviews

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

Review

Labelling proteins and peptides with phosphorescent d^6 transition metal complexes

Timothy U. Connell [a], Paul S. Donnelly [b,*]

[a] School of Science, RMIT University, Melbourne, VIC 3001, Australia
[b] School of Chemistry and Bio21 Molecular Science and Biotechnology Institute, University of Melbourne, Melbourne 3010, Australia

ARTICLE INFO

Article history:
Received 9 October 2017
Received in revised form 1 December 2017
Accepted 1 December 2017
Available online xxxx

ABSTRACT

The phosphorescent properties of Ru(II), Re(I) and Ir(III) transition metal complexes, each with a nominal d^6 electron configuration, are well suited to label peptides and proteins to allow biological imaging by luminescent microscopy. This review focuses on the development of chemistry that enables the ready incorporation of a wide range of phosphorescent metal complexes into peptides or proteins. Different strategies for labelling proteins are presented including the direct coordination of amino acid functional groups to the metal ion, metal complexes where one or more of the ligands coordinated to the metal ion contain reactive functional groups for conjugation to peptides or proteins, and metal complexes designed for site selective bioorthogonal conjugation. Phosphorescent metal complexes that bind and interact with proteins in a non-covalent fashion are also discussed as is the use of metal complexes as single-electron transfer photo-catalysts for the fluorescent labelling of proteins.

Contents

Abbreviations: Aβ, amyloid-β; BRD4, bromodomain-containing protein 4; BSA, bovine serum albumin; COX-2, cyclooxygenase-2; *D/P*, dye:protein ratio, used to quantify the number of metal complexes attached per protein; DNA, deoxyribonucleic acid; EC_{50}, half maximal effective concentration, used primarily when discussing cellular toxicity of metal complexes, particular in photo-dynamic therapy applications; ELISA, enzyme-linked immunosorbent assay; ER, estrogen receptor protein; FRET, Förster resonance energy transfer; GSH, glutathione; HSA, human serum albumin; I/I_0, emission enhancement number, used to quantify the increase in emission following a specific interaction; IC_{50}, half maximal inhibitory concentration, used primarily when discussing protein target inhibition of metal complexes; MLCT, metal–ligand charge transfer; NF-κB, nuclear factor-κB; NLS, nuclear localisation signal; *Pf*HRPII, *Plasmodium falciparum* histidine-rich protein II; RNA, ribonucleic acid; SAAC, single amino acid chelate; SPECT, single-photon emission computed tomography; SPPS, solid phase peptide synthesis; STAT3, signal transducer and activator of transcription protein 3; TACE, TNF-α converting enzyme; TAT, *trans*-activating transcriptional activator; Tf, transferrin; TNF-α, tumour necrosis factor α; VDAC1, voltage-dependent anion-selective channel 1.

* Corresponding author.
E-mail address: pauld@unimelb.edu.au (P.S. Donnelly).

https://doi.org/10.1016/j.ccr.2017.12.001
0010-8545/

1. Introduction

Proteins are central to living organisms and perform a myriad of functions as diverse as catalysis, molecular transport, generation of movement and nerve impulses as well as governing the responses to internal and external stimuli. Fluorescent imaging is often used in combination with immunological staining to probe the molecular detail of proteins particularly in cell cultured based experiments. The development of super-resolution techniques has improved sensitivity and resolution to the point that single molecule detection is increasingly routine [1]. The timescale of protein function varies from seconds to several days and traditional organic fluorophores such as fluorescein, rhodamine and cyanine dye derivatives are susceptible to photo-bleaching so are often not ideal for following processes such as protein trafficking. Phosphorescent transition metal complexes, particularly those with a d^6 electron configuration, exhibit unique photo-physical properties that have seen them emerge as attractive alternative to organic dyes. The origin of these phenomena is covered in other reviews [2,3], but those most influential for labelling biomolecules include: (i) the possibility to tune photo-physical properties of the complexes to obtain emission energies that span the visible electromagnetic spectrum by altering the ligand environment (common ligands and their abbreviations are highlighted in Fig. 1) around the metal ion. (ii) Synthetic strategies are well defined to incorporate functional groups into the metal complexes that permit incorporation of biological molecules, peptides or proteins. (iii) Strong spin–orbit coupling from the heavy metal atom promotes triplet excited state emission which occurs with large Stokes shifts with minimal loss of signal through self-quenching and Förster resonance energy transfer (FRET) pathways. (iv) The long emission lifetimes of the triplet based emission enable increased time-gated imaging to remove background fluorescence and give increased sensitivity. (v) Metal based luminophores are often more resistant to photobleaching than organic dyes so permit imaging over longer periods of time before loss of fluorescence signal. (vi) Phosphorescent metal complexes are relatively small in size with low molecular weights so impart minimal effect on the distribution and function of the protein target especially when compared to semiconductor quantum dots or encoded fluorescent proteins such as green fluorescent protein.

This review focuses on the use of either iridium(III), rhenium(I) or ruthenium(II) phosphorescent complexes, with a nominal d^6 electron configuration, to label proteins. Metal complexes are also able to bind and interact with proteins in a non-covalent fashion and a brief summary of transition metal complexes employed in this manner is presented as is their use as single-electron transfer photo-catalysts to promote fluorescent labelling of proteins. Pioneering work is mentioned when appropriate, but the emphasis is placed on research reported post 2010. The use of phosphorescent metal complexes as cellular organelle specific stains and small molecule sensors is well covered in other excellent reviews [4–9].

2. Labelling proteins with phosphorescent d^6 metal complexes

2.1. Attaching peptides to phosphorescent metal complexes by exploiting coordinate bond formation with amino acid residues

Solvated bis-cyclometallated iridium complexes, such as $[Ir(\mathbf{ppy})_2(solv)_2]^+$ where solv is either coordinated water, acetonitrile or dimethylsulfoxide (Fig. 2), are typically non-emissive but readily form phosphorescent complexes upon reaction with histidine residues in peptides and proteins. The imidazole functional group replaces the readily exchangeable solvent ligands. The emission response of the iridium(III) complexes **1** and **2** upon coordination to proteins following gel electrophoresis enabled visualization with the added advantage, when compared to conventional staining with Coomassie brilliant blue, that there is no need to perform a destaining process [10]. Optimal binding requires either a di-histidine sequence or a single histidine residue located at the N-terminus [11]. A derivative of a cell penetrating TAT-based peptide (TAT = *trans*-activating transcriptional activator), with the sequence H_2N-**H**RKKRRQRRR-CO_2H (using conventional single letter codes for different amino acid) containing an N-terminal histidine residue was labelled by a reaction with $[Ir(\mathbf{ppy})_2(CH_3CN)_2]^+$, **2**. The resulting phosphorescent complex crosses the cell membrane in HeLa cervical cancer cells allowing visualization by confocal luminescent microscopy.

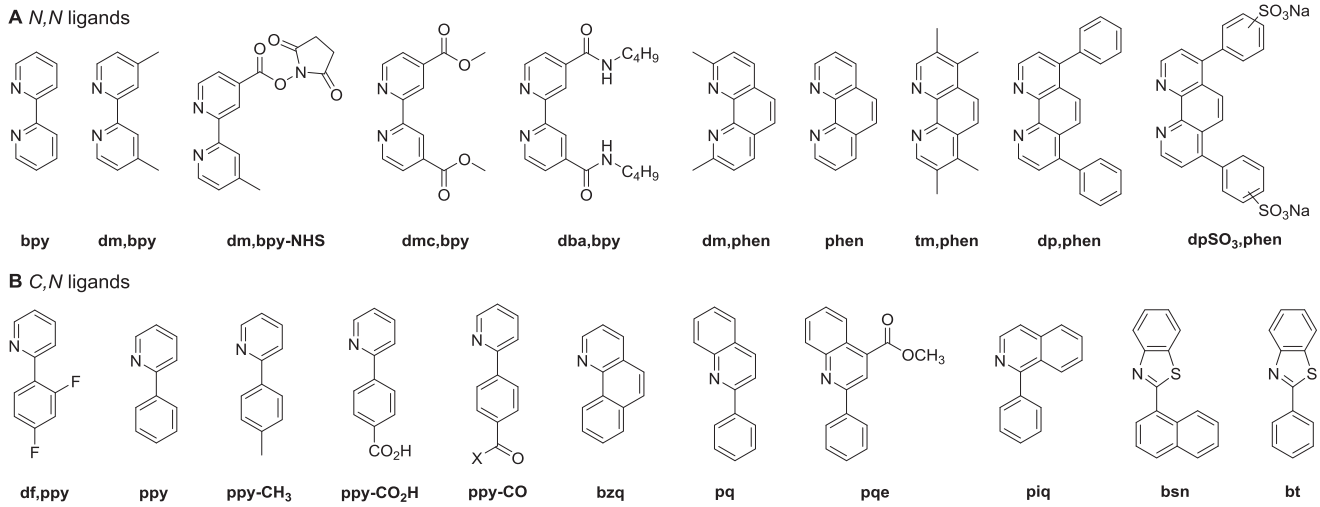

Fig. 1. Common coordinating ligands found in phosphorescent metal complexes.

Please cite this article in press as: T.U. Connell, P.S. Donnelly, Labelling proteins and peptides with phosphorescent d^6 transition metal complexes, Coord. Chem. Rev. (2017), https://doi.org/10.1016/j.ccr.2017.12.001