Biochimica et Biophysica Acta 1828 (2013) 2319–2327

Contents lists available at SciVerse ScienceDirect

Biochimica et Biophysica Acta

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

Review

Coordination to divalent cations by calcium-binding proteins studied by FTIR spectroscopy☆

Masayuki Nara [a,*], Hisayuki Morii [b], Masaru Tanokura [c]

[a] Laboratory of Chemistry, College of Liberal Arts and Sciences, Tokyo Medical and Dental University, Chiba 272-0827, Japan
[b] National Institute of Advanced Industrial Science and Technology (AIST), Ibaraki, Japan
[c] Department of Applied Biological Chemistry, Graduate School of Agricultural and Life Sciences, University of Tokyo, Bunkyo, Tokyo, Japan

ARTICLE INFO

Article history:
Received 17 October 2012
Received in revised form 15 November 2012
Accepted 17 November 2012
Available online 29 November 2012

Keywords:
FTIR spectroscopy
COO^- group
Calcium binding protein
Coordination structure

ABSTRACT

We review the Fourier-transform infrared (FTIR) spectroscopy of side-chain COO^- groups of Ca^{2+}-binding proteins: parvalbumins, bovine calmodulin, akazara scallop troponin C and related calcium binding proteins and peptide analogues. The COO^- stretching vibration modes can be used to identify the coordination modes of COO^- groups of Ca^{2+}-binding proteins to metal ions: bidentate, unidentate, and pseudo-bridging. FTIR spectroscopy demonstrates that the coordination structure of Mg^{2+} is distinctly different from that of Ca^{2+} in the Ca^{2+}-binding site in solution. The interpretation of COO^- stretches is ensured on the basis of the spectra of calcium-binding peptide analogues. The implication of COO^- stretches is discussed for Ca^{2+}-binding proteins. This article is part of a Special Issue entitled: FTIR in membrane proteins and peptide studies.

Contents

1. Introduction

Fourier-transform infrared spectroscopy (FTIR) is a useful method for investigating protein structures [1–8]. Among the infrared bands created by the peptide group, the amide-I and amide-I′ bands have been the most widely used in studies of protein secondary structures in H_2O and D_2O solutions, respectively. The amide-I mode consists mainly of the C O stretch of the peptide group (mixed with the N–H bend and the C–N stretch) and gives rise to a strong infrared band in the region of 1700–1600 cm^{-1}. The amide-I′ mode also consists mainly of the C O stretch of the peptide group, but the band position of amide-I′ mode is very slightly downshifted due to the secondary order of perturbation by the HD exchange at NH bond of the main chain. The development of FTIR spectroscopy made it possible to enhance the resolution of broad infrared bands by techniques such as Fourier self-deconvolution [9–11], second-derivative, curve-fitting, difference calculation and two-dimensional correlation analysis [12–14]. Originally, the relationship between the positions of the amide-I band obtained by using Fourier self-deconvolution and curve-fitting and the type of secondary structure was investigated experimentally for model peptides and proteins of known three-dimensional structure by Byler and Susi [3]. The general empirical rule in the infrared study of proteins is to assign the individual amide-I bands resolved by resolution-enhancement techniques to representative secondary structures such as α-helix, β-sheet, β-turn and so on [1–8]. In particular, to understand the secondary structures of proteins

Abbreviations: Parv(s), parvalbumin(s); CaM, calmodulin; TnC, troponin C

☆ This article is part of a Special Issue entitled: FTIR in membrane proteins and peptide studies.

* Corresponding author. Tel.: +81 47 300 7122; fax: +81 47 300 7100.
E-mail address: nara.las@tmd.ac.jp (M. Nara).

0005-2736/$ – see front matter © 2012 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.bbamem.2012.11.025

qualitatively, second-derivative calculation has been widely and conveniently applied to the FTIR spectra or FTIR attenuated total reflection (ATR) spectra of proteins, after eliminating the contribution of solvent (H_2O or D_2O buffer) by a subtraction procedure.

FTIR spectroscopy also has potential in the study of protein side-chains such as aromatic rings, $-COO^-$, $-OH$, $-SH$, $-CH_3$, $-CH_2-$ and so on, to elucidate the mechanisms underlying protein reactions [7,8,15]. Wright and Vanderkooi indicated that FTIR profiles for 20 amino acids and their metabolites are sufficiently characteristic so that FTIR can be used to monitor enzymatic reactions involving amino acids [15]. Barth has reviewed the infrared absorption of amino acid side chains of proteins in H_2O and D_2O in detail [7,8]. In this review, we focus on the metal coordination of the side chain COO^- groups of Glu and Asp on Ca^{2+}-binding proteins, which plays an important role in Ca^{2+}-mediated functions [16–27].

2. Implication of infrared COO^- stretches

The carboxylate (COO^-) groups can coordinate to metal ions in four modes (Fig. 1): 'unidentate' (or 'monodentate'), 'bidentate' (or 'chelating'), 'bridging' (or 'bridging bidentate') and 'pseudo-bridging' modes [28,29]. When a metal ion interacts with only one oxygen atom of a COO^- group, the coordination structure is regarded as unidentate. In the bidentate coordination mode, the metal ion interacts equally with the two oxygen atoms of a COO^- group. In the bridging coordination mode, one metal ion binds to one of the two oxygens in a COO^- group and another metal ion to the other oxygen atom. As a special case of the bridging mode, the pseudo-bridging coordination mode features a water molecule replacing one of the two ligands in the bridging coordination. Extensive infrared studies have been done on the relationship between COO^- stretching frequencies and coordination types [28,30]. Deacon and Phillips [28] have found a general tendency in the relationship between $\Delta\nu_{a-s}$ (frequency separation between the COO^- antisymmetric and symmetric stretching vibrations) and the coordination types of the COO^- group to metal ions by examining the structures and vibrational frequencies of a number of acetate salts in the solid state. The frequency of the COO^- antisymmetric stretch of the unidentate species is higher than that of the ionic (metal-free) species, which is in turn higher than that of the bidentate species. The reverse is the case for the COO^- symmetric stretch. As a result, the $\Delta\nu_{a-s}$ values for unidentate, bridging, bidentate and ionic species are in the following order:

$$\Delta\nu_{a-s}(\text{unidentate}) > \Delta\nu_{a-s}(\text{ionic}) \sim \Delta\nu_{a-s}(\text{bridging}) > \Delta\nu_{a-s}(\text{bidentate}),$$

where $\Delta\nu_{a-s}$ (ionic) is approximately 160–170 cm^{-1}. *Ab initio* molecular orbital calculation ($HF/6-31+G^{**}$) has revealed that the correlation is related to changes in the CO bond length and the OCO angle [31]. An equation for the relationship between the structure of the COO^- group and the value of $\Delta\nu_{a-s}$ (in cm^{-1}) is given as

$$\Delta\nu_{a-s} = 1818.1\delta r + 16.47(\theta_{OCO} - 120) + 66.8,$$

where δr is the difference between the two CO bond lengths (in Å) and θ_{OCO} is the OCO angle (in degrees). This equation suggests that the variation of 0.01 Å in δr or 1° in θ_{OCO} gives rise to a change of 16–18 cm^{-1} in the value of $\Delta\nu_{a-s}$. Dudev and Lim have evaluated vibrational frequencies and absolute intensities of the COO^- stretches by using density functional theory (DFT) calculation and suggested that IR band intensities may be used to help interpret the IR spectra of protein binding sites in the metal-free and metal-bound states [32].

The empirical rule described above can be applied to other compounds, such as amino acids (glutamic and aspartic) and ethylenediaminetetraacetic acid (EDTA), although the value $\Delta\nu_{a-s}$ (ionic) depends on the compound. When we apply this empirical rule to the side-chain COO^- groups contained in a protein in solution, we see that the COO^- group, which binds to M^{2+} (bicationic metal ion) in the unidentate coordination mode in the solid state, probably contacts water molecules in aqueous solution and may become a 'pseudo-bridging' coordination mode. This is applicable to [$EDTA^{4-}$–Ca^{2+}] complex in aqueous solution [20] and most of the side chain COO^- groups in the unidentate coordination mode in Ca^{2+}-binding proteins. As a result of the coordination of the COO^- groups to Ca^{2+} in the pseudo-bridging mode, the intensity of the COO^- antisymmetric stretching band becomes stronger by the binding of Ca^{2+}, not apparent in the case of the COO^- symmetric stretching band [20].

The band positions of COO^- stretches due to the β-COO^- group of Asp and the γ-COO^- group of Glu can be applied to the side chains of the COO^- groups of Asp and Glu of proteins, respectively: Asp $\nu_{as}(COO^-)$ 1584 cm^{-1}, $\nu_s(COO^-)$ 1402 cm^{-1} and Glu $\nu_{as}(COO^-)$ 1567 cm^{-1}, $\nu_s(COO^-)$ 1407 cm^{-1} in D_2O solution [7]. Usually, the behavior of COO^- symmetric stretch can be investigated by using protein samples in H_2O solution, where the handling of HD exchange in sample solution is not necessary. However, it is difficult to obtain information about the behavior of the COO^- antisymmetric stretch in H_2O solution, because the COO^- antisymmetric stretching band overlaps with the amide II band. Therefore, to obtain reliable infrared spectra in the region of COO^- antisymmetric stretch, exchangeable protons in the protein should be completely deuterated by incubating the apo protein dissolved in D_2O in mild heating condition (e.g. for 60 min at 60 °C). If it is difficult to exchange H for D completely for amide groups of membrane proteins and biological systems, it is suitable to analyze the COO^- symmetric stretch alone. For example, the information about the coordination structures of Ca^{2+} and Mn^{2+} has been successfully obtained for photosynthetic oxygen-evolving center [33,34].

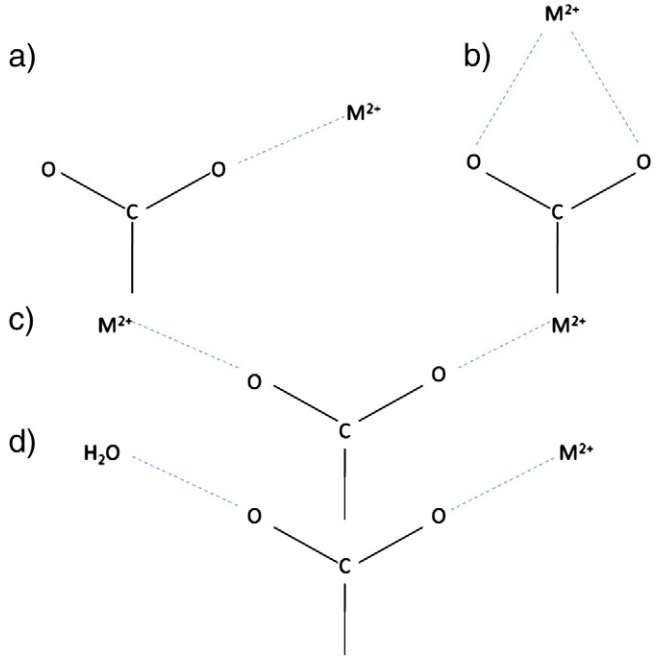

Fig. 1. Coordination structures of the side chain COO^- groups to M^{2+} in (a) unidentate, (b) bidentate, (c) bridging and (d) pseudo-bridging modes.

3. Parvalbumins

Parvalbumins (Parvs), which are ubiquitous in vertebrates, form a group in Ca^{2+}-binding proteins in parallel with calmodulin (CaM)