FEBS Letters 349 (1994) 84–88

FEBS 14259

Infrared studies of interaction between metal ions and Ca^{2+}-binding proteins

Marker bands for identifying the types of coordination of the side-chain COO^- groups to metal ions in pike parvalbumin (pI = 4.10)

Masayuki Nara[a], Mitsuo Tasumi[a,*], Masaru Tanokura[b], Toshifumi Hiraoki[c], Michio Yazawa[d], Akihiro Tsutsumi[c]

[a]*Department of Chemistry, School of Science, The University of Tokyo, Bunkyo-ku, Tokyo 113, Japan*
[b]*Biotechnology Research Center, The University of Tokyo, Bunkyo-ku, Tokyo 113, Japan*
[c]*Department of Applied Physics, Faculty of Engineering, Hokkaido University, Kita-ku, Sapporo 060, Japan*
[d]*Department of Chemistry, Faculty of Science, Hokkaido University, Kita-ku, Sapporo 060, Japan*

Received 20 May 1994

Abstract

Metal–ligand interactions in the Ca^{2+}-binding sites of pike parvalbumin (pI = 4.10) have been examined by Fourier-transform infrared spectroscopy. The region of the COO^- antisymmetric stretch provides useful information on the types of coordination of the COO^- groups to the metal ions in the Mg^{2+}-, Mn^{2+}-, and Ca^{2+}-bound forms. In the spectrum of the Ca^{2+}-bound form, two bands are observed at 1,582 and 1,553 cm^{-1}, whereas, in the spectra of the Mg^{2+}- and Mn^{2+}-bound forms, bands are observed only in the region around 1,582 cm^{-1} and no band is found in the region around 1,553 cm^{-1}. The 1,553-cm^{-1} band of the Ca^{2+}-bound form reflects the bidentate coordination of the COO^- groups of both Glu-62 in the CD site and Glu-101 in the EF site to the Ca^{2+} ions, which has been made clear by X-ray analysis as a feature of the Ca^{2+}-bound form. Absence of such a band in the spectrum of the Mn^{2+}-bound form is consistent with the X-ray structure of this form where both of the two COO^- groups are unidentate. These unidentate COO^-groups of Glu-62 and Glu-101 in the Mn^{2+}-bound form seem to give rise to a band at 1,577–1,574 cm^{-1}. The spectrum of the Mg^{2+}-bound form is also consistent with the 'pseudo-bridging' coordination of the COO^- group of Glu-101 reported in the X-ray structure of a form where the Mg^{2+} ion occupies only the EF site, and the same spectrum is further indicative of the 'pseudo-bridging' coordination of the COO^- group of Glu-62.

Key words: Pike parvalbumin; Ca^{2+}-binding protein; Metal–ligand interaction; Infrared spectroscopy; COO^- antisymmetric stretch; Coordination type

1. Introduction

Parvalbumins which are ubiquitous in vertebrates form a group in Ca^{2+}-binding proteins [1] in parallel with calmodulin [2–5] and troponin C [6,7]. Although the function of parvalbumins has not been fully understood yet, it has been proposed [8,9] that they are involved in the relaxation process of fast muscles. Kretsinger and Nockolds [10] first reported the three-dimensional structure of carp parvalbumin (isoform pI = 4.25) in crystal. According to their result, which was later refined by Moews and Kretsinger [11], this protein has a globular shape containing six helical parts called the A~F helices from the N-terminus, and has a feature common to Ca^{2+}-binding proteins, namely, the EF-hand conformation, which is formed by 30 amino acid residues consisting of the E and F helices (nearly perpendicular to each other) and a connecting loop with a Ca^{2+}-binding site (EF site). Another domain of 30 amino acid residues containing the C and D helices also assumes a similar conformation with a Ca^{2+}-binding site in it (CD site). Such general characteristics in the three-dimensional structure have been confirmed by other X-ray analyses [12–16] and nuclear magnetic resonance studies [17,18] of parvalbumins from various sources.

A more recent X-ray analysis of carp parvalbumin at higher resolution (1.5 Å) by Kumer et al. [15] has shown that the previous result obtained by Moews and Kretsinger [11] should be corrected with regard to the type of coordination of the carboxylate groups to the Ca^{2+} ions. According to the result presented by Kumer et al. [15], the Ca^{2+} ions in both the CD and EF sites are 7-coordinate; the ligands in the CD site are Asp-51, Asp-53, Ser-55 (O of the OH group), Phe-57 (O of the main-chain CO group), Glu-59, and Glu-62 and those in the EF site are Asp-90, Asp-92, Asp-94, Lys-96 (O of the main-chain CO group), Glu-101, and water-128. The COO^- groups of all the above aspartic acid residues and

*Corresponding author. Fax: (81) (3) 3815-8684 *or* (81) (3) 3814-2627.

SSDI 0014-5793(94)00645-C

Glu-59 are unidentate, whereas those of Glu-62 and Glu-101 are bidentate.

The Mg^{2+} and Mn^{2+} ions have affinities for the Ca^{2+}-binding sites in parvalbumins, but the association constants for Mg^{2+} are 3–4 orders of magnitude smaller than those for Ca^{2+} [19, 20]. Pike parvalbumin (pI = 4.10) is particularly interesting for the purpose of studying the metal-ligand interactions in Ca^{2+}-binding proteins, because Declercq et al. [13,16] have reported the X-ray structures (1.6–1.8 Å resolution) of this protein for not only the Ca^{2+}-bound form but also the Mn^{2+}-bound form and a partially Mg^{2+}-bound form where the Mg^{2+} ion is bound only to the EF site. The primary structures of the two Ca^{2+}-binding sites in pike parvalbumin (pI = 4.10) are exactly the same as those of carp parvalbumin (pI = 4.25), and the X-ray structures of the Ca^{2+}-binding sites in the Ca^{2+}-binding forms of these two kinds of parvalbumins are essentially the same. In contrast with the Ca^{2+}-bound form, the COO^- groups of both Glu-62 and Glu-101 in the Mn^{2+}-bound form of pike parvalbumin are unidentate. The COO^- group of Glu-101 in the partially Mg^{2+}-bound form is also unidentate, or more precisely, in the pseudo-bridging state as will be described later.

The first three of the present authors have been trying to study by infrared spectroscopy the metal–ligand interactions in Ca^{2+}-binding proteins, or more generally speaking, effects of metal ions on the three-dimensional structures of this class of proteins [21,22]. The purpose of the present paper is to show that comparison of the infrared spectra of the metal-bound forms (metal = Mg^{2+}, Mn^{2+}, and Ca^{2+}) of pike parvalbumin (pI = 4.10) leads to a unique identification of bands which can be used as markers for the type of coordination of the COO^- group to the metal ion. Such marker bands are expected to be useful for studying the metal– ligand interactions in other Ca^{2+}-binding proteins as well.

2. Materials and methods

Ca^{2+}-free parvalbumin (pI = 4.10) (powder) was prepared from pike (*Esox lucius*) skeletal white muscle by the method described previously [23,24]. Contaminating Ca^{2+} was removed by treatment with trichloroacetic acid [24]. To obtain reliable infrared spectra in the regions of the COO^- antisymmetric stretch and the amide-I′ mode, exchangeable protons in the protein were completely deuterated in the following way. The Ca^{2+}-free protein dissolved in D_2O was incubated at 60°C for 30 min. After cooling the solution to room temperature, the solution was freeze-dried. The concentrations of sample solutions of the metal-bound proteins for infrared measurements were adjusted to 4 mM. Sample solutions of the Mn^{2+}- and Mg^{2+}-bound proteins were obtained by dissolving the powder of deuterated Ca^{2+}-free protein in a D_2O solution containing 0.2 M $MnCl_2$ or $MgCl_2$ in addition to 0.1 M KCl and 25 mM HEPES buffer (pD 7.5).

Infrared measurements were carried out at room temperature on a Jeol JIR 5,500 Fourier-transform infrared spectrophotometer at 2 cm^{-1} resolution. Interferograms from 1,000 scans were averaged to obtain one spectrum. Dry air was constantly flowed into the spectrophotometer during spectral measurements. About 0.012 ml of the sample (D_2O solution obtained above) was set between two CaF_2 plates using a 0.015 mm thick Teflon spacer. The gap between the two CaF_2 plates was sealed with aluminium tape to suppress evaporation of water. Infrared spectra of the solvent (buffer solution) were measured in the same way. To eliminate the contribution of absorptions due to D_2O from the spectrum of the protein solution, the spectrum of the solvent was subtracted, after multiplying an appropriate factor, from the spectrum of the protein solution.

Since the observed infrared bands of the proteins in aqueous solution were very broad, the techniques of resolution enhancement, namely, second-derivative calculation and Fourier-self-deconvolution were applied to the observed spectra to extract useful information hidden in the broad bands [25–27]. Second-derivative calculation was performed by the software supplied by Jeol Co. Deconvolution was performed according to the method described by Jones and Shimokoshi [28]. A Lorentz bandshape function and a (triangle)^2 apodizing function were used with the following parameters: 2σ = 28 cm^{-1} and L = 0.18 cm.

3. Results

The infrared spectra (1,800–1,300 cm^{-1}) of the metal-bound forms of deuterated pike parvalbumin (pI = 4.10) are shown in Fig. 1, where absorptions of D_2O have already been subtracted. In each spectrum in Fig. 1, four broad bands are seen, namely, the amide-I′ band at about 1,645 cm^{-1}, the COO^- antisymmetric stretching band at about 1,583 cm^{-1}, the amide-II′ band at about 1,454 cm^{-1}, and the COO^- symmetric stretching band at about 1,402 cm^{-1}.

The three spectra in Fig. 1 are similar to each other, but significant differences are observed in the region of the COO^- antisymmetric stretch; the band at 1,581 cm^{-1} of the Mn^{2+}-bound form in Fig. 1b seems to be slightly broader than the band at 1,583 cm^{-1} of the Mg^{2+}-bound form in Fig. 1a, and the weak band at 1,553 cm^{-1} in Fig. 1c is seen only for the Ca^{2+}-bound form.

The spectra in Fig. 1 are deconvolved and shown in Fig. 2, together with the corresponding second-derivative spectra. Both the deconvolved spectra and second-derivative spectra confirm the above observations in the region of 1,600–1,540 cm^{-1}, in that there are actually two bands at 1,584 and 1,577 cm^{-1} in Fig. 2c (1,585 and 1,574 cm^{-1} in Fig. 2d), while there is a single band at 1,584 cm^{-1} in Fig. 2a and b and at 1,582 cm^{-1} in Fig. 2e and f, and the band at 1,553 cm^{-1} in Fig. 2e and f is characteristic of the Ca^{2+}-bound form. In each spectrum in Fig. 2, a weak band is found at about 1,605–1,603 cm^{-1}. These observations will be discussed in detail in section 4.

In the region of the COO^- symmetric stretch, the spectra of the Mn^{2+}-bound form (Fig. 2c and d) and the Ca^{2+}-bound form (Fig. 2e and f) are close to each other, having two bands at 1,422–1,420 and 1,400–1,397 cm^{-1}, but the spectra of the Mg^{2+}-bound form (Fig. 2a and b) are slightly different with a band at about 1,424 cm^{-1} which appears to be broader than the bands at 1,422–1,420 cm^{-1} in Fig. 2c–f.

In the amide-I′ region also, the spectra of the Mn^{2+}- and Ca^{2+}-bound forms are close to each other, but the spectra of the Mg^{2+}-bound form are slightly different

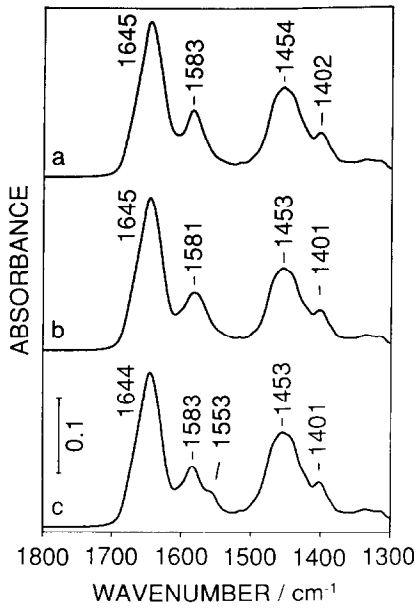

Fig. 1. Infrared spectra of the Mg^{2+}-bound (a), Mn^{2+}-bound (b), and Ca^{2+}-bound (c) forms of pike parvalbumin in D_2O solution.

from those of the other two. The wavenumber of the most intense peak of the Mg^{2+}-bound form (1,649 cm^{-1}) is apparently higher than those of the Mn^{2+}- and Ca^{2+}-bound forms (1,646 and 1,644 cm^{-1}, respectively).

4. Discussion

As described in section 3, four groups of bands due to the amide-I′, COO^- antisymmetric stretch, amide-II′, and COO^- symmetric stretch are observed in Figs. 1 and 2, in addition to some weaker bands whose origins are not clearly identified at present. In this paper, we focus our attention on bands in the region of the COO^- antisymmetric stretch [$\nu_{as}(COO^-)$], since they provide us with useful information on the metal–ligand interaction in pike parvalbumin and other Ca^{2+}-binding proteins as well.

It is well known [29] that the COO^- group in aqueous media gives rise to a strong infrared absorption at about 1,600 cm^{-1} due to the antisymmetric stretch. The results of our own measurements on sodium aspartate and sodium glutamate in various conditions are as follows. The β-COO^- group of aspartate in neutral D_2O solution (0.2 M) gives rise to a band at 1,584 cm^{-1}, and the γ-COO^- group of glutamate in the same condition a band at 1,567 cm^{-1}. With the addition of $CaCl_2$ to the above solution in a molar ratio of five ($CaCl_2$) to one (aspartate or glutamate), neither the 1,584-cm^{-1} band of aspartate nor the 1,567-cm^{-1} band of glutamate shows an appreciate change, probably because the association constant between Ca^{2+} and the free aspartate or glutamate anion is small. For paste-like samples obtained with addition of large excesses of $CaCl_2$, however, the 1,584-cm^{-1} band of aspartate shows a large downshift to 1,547 cm^{-1}, and the 1,567-cm^{-1} band of glutamate to 1,551 cm^{-1}.

Deacon and Phillips [30] have found a general trend in the relationship between the position of the $\nu_{as}(COO^-)$ band and the type of coordination of the COO^- group to divalent metal cations by examining a number of data observed for the acetate anion in the solid state. The general trend may be summarized as follows. (1) Bidentate coordination of the COO^- group to a divalent metal cation downshifts the position of the $\nu_{as}(COO^-)$ band from that of the COO^- group not interacting strongly with a metal cation (called the 'ionic' carboxylate group), as exemplified by the COO^- group in solid sodium acetate. (2) Unidentate coordination of the COO^- group to a divalent metal cation upshifts the $\nu_{as}(COO^-)$ band from the position of the 'ionic' $\nu_{as}(COO^-)$ band. (3) In the 'bridging' coordination where one divalent metal cation is bound to one of the two oxygens in the COO^- group and another divalent metal cation to the other oxygen, the $\nu_{as}(COO^-)$ band is located at essentially the same position as that of the 'ionic' $\nu_{as}(COO^-)$ band. (4) In the 'pseudo-bridging' coordination also, where one divalent metal cation is bound to one of the two oxygens in the

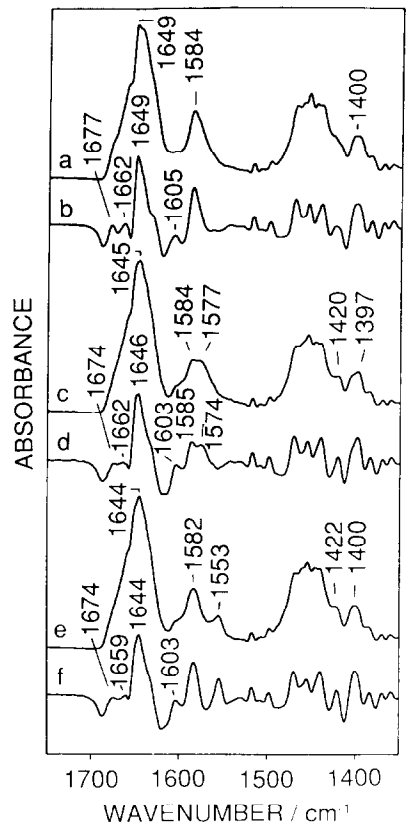

Fig. 2. Fourier-self-deconvolved (a, c, e) and second-derivative spectra (b,d,f) of the Mg^{2+}-bound (a,b), Mn^{2+}-bound (c,d), and Ca^{2+}-bound (e,f) forms of pike parvalbumin. Second derivatives are multiplied by −1.

COO^- group and a water molecule is hydrogen-bonded to the other oxygen, the position of the $\nu_{as}(COO^-)$ band is close to that of the 'ionic' $\nu_{as}(COO^-)$ band.

On the basis of the above observations on the $\nu_{as}(COO^-)$ bands of the model compounds, the bands observed in the region of 1,610–1,550 cm^{-1} in Figs. 1 and 2 may be correlated to the local environments of the COO^- groups in the protein molecule.

(a) The bands at 1,584–1,582 cm^{-1} in Fig. 2 correspond exactly in position to the 1584-cm^{-1} band of 'free' aspartate in neutral D_2O solution. Pike parvalbumin has 14 aspartic acid residues in total and five of them are located in the CD and EF Ca^{2+}-binding sites. Most probably, at least some of the COO^- groups of seven aspartic acid residues located outside the Ca^{2+}-binding sites and two in the Ca^{2+}-binding sites (not coordinated to the metal ion) can interact with the solvent in the same way as the 'free' aspartate anion does, and give rise to the bands at 1,584–1,582 cm^{-1}.

(b) The band at 1,553 cm^{-1} of the Ca^{2+}-bound form in Fig. 1c, and Fig. 2e and f is undoubtedly due to the COO^- groups of Glu-62 and Glu-101 which are coordinated to Ca^{2+} in the bidentate mode. The fact that this band is characteristic of the Ca^{2+}-bound form agrees completely with the results of X-ray analyses [16] that the COO^- groups of Glu-62 and Glu-101 are bidentate only in the Ca^{2+}-bound form. The 1,553-cm^{-1} band of the Ca^{2+}-bound form is 14 cm^{-1} downshifted from the 1,567-cm^{-1} band of 'free' glutamate in neutral D_2O solution. This downshift parallels that of the $\nu_{as}(COO^-)$ band of the acetate anion on going from the 'ionic' state to the bidentate state. Furthermore, the position of the 1,553-cm^{-1} band of the Ca^{2+}-bound form coincides with that of the 1,551-cm^{-1} band of sodium glutamate in the presence of an excess of $CaCl_2$, although there is no other evidence for bidentate coordination of the glutamate γ-COO^- group in the paste-like sample.

(c) The band at 1,577–1,574 cm^{-1} in Fig. 2c and d is probably due to the COO^- groups of Glu-62 and Glu-101 in the Mn^{2+}-bound form, which are unidentate according to the result of X-ray analysis [16]. This band is 7–10 cm^{-1} upshifted from the 1,567-cm^{-1} band of 'free' glutamate in parallel with the upshift of the $\nu_{as}(COO^-)$ band of the acetate anion on going from the 'ionic' state to the unidentate state.

(d) According to the X-ray analysis of the partially Mg^{2+}-bound form [16], the COO^- group of Glu-101 in this form is in the pseudo-bridging state. The band at 1,584 cm^{-1} of the Mg^{2+}-bound form in Fig. 2a and b may contain a contribution from $\nu_{as}(COO^-)$ of Glu-101 in the pseudo-bridging state, in addition to the absorption due to the 'free' COO^- groups of aspartic acid residues. Even if the association constant for Mg^{2+} is 3–4 orders of magnitude smaller than that for Ca^{2+}, both of the two Ca^{2+}-binding sites are considered to be occupied by Mg^{2+} in the Mg^{2+}-bound form, because the concentration of Mg^{2+} is sufficiently higher than that of the protein in the present experimental condition. The spectra in Fig. 2a and b seem to indicate that the COO^- group of Glu-62 in the Mg^{2+}-bound form is also in the pseudo-bridging state. If it is in either the bidentate or unidentate state, it should give rise to a band in Fig. 2a and b either at about 1,553 cm^{-1} or at about 1,577–1,574 cm^{-1}, by analogy with the case described in (b) or (c), respectively.

(e) It is more difficult to identify the origin of the weak band at 1,605–1,603 cm^{-1} observed in all the spectra in Fig. 2. However, it may be worth mentioning the following possibilities. (1) The COO^- groups of the aspartic acid residues in the Ca^{2+}-binding sites are in the pseudo-bridging state. These COO^- group may give rise to the band at 1,605–1,603 cm^{-1}. (2) Some of the aspartic acid and glutamic acid residues located outside the Ca^{2+}-binding sites may form a hydrogen bond involving one of the two oxygens in the COO^- group. In such a case, the stretch of the non-hydrogen-bonded CO bond may give rise to the band at 1,605–1,603 cm^{-1}.

In conclusion, it should be emphasized that Fourier-transform infrared spectroscopy combined with the techniques of resolution enhancement may provide a powerful tool for studying metal–ligand interactions in Ca^{2+}-binding proteins in general and possibly in other proteins as well. Careful comparisons of the information obtainable for other Ca^{2+}-binding proteins from infrared spectroscopy with their high-resolution X-ray structures are most desirable.

Acknowledgements: We thank the Ueno Zoological Garden Aquarium for the gift of a pike.

References

[1] Pechère, J.F. (1977) Calcium-binding Protein and Calcium (Wasserman, R.H., Corradino, R., Carafoli, E., Kretsinger, R.H., MacLennan, D.H. and Siegel, F.L. eds.) pp. 213–221, Elsevier, New York.
[2] Klee, C.B., Crouch, T.H. and Richman, P.G. (1980) Annu. Rev. Biochem. 49, 489–515.
[3] Klee, C.B. and Vanaman, T.C. (1982) Adv. Protein Chem. 35, 213–321.
[4] Means, A.R., Tash, J.S. and Chafouleas, J.G. (1982) Physiol. Rev. 62, 1–39.
[5] Cox, J.A. (1988) Biochem. J. 249, 621–629.
[6] Cheung, H.C., Wang, C.K. and Garland, F. (1982) Biochemistry 21, 5135–5142.
[7] Ohtsuki, I., Maruyama, K. and Ebashi, S. (1986) Adv. Protein Chem. 38, 1–67.
[8] Gills, J.M., Thomason, D., Lefèvre, J. and Kretsinger, R.H. (1982) J. Muscle Res. Cell Motil. 3, 377–398.
[9] Heizmann, C.W., Berchtold, M.W. and Rowlerson, A.M. (1982) Proc. Natl. Acad. Sci. USA 79, 7243–7247.
[10] Kretsinger, R.H. and Nockolds, C.E. (1973) J. Biol. Chem. 248, 3313–3326.
[11] Moews, P.C. and Kretsinger, R.H. (1975) J. Mol. Biol., 91, 201–228.
[12] Wéry, J.P., Dideberg, O., Charlier, P. and Gerday, C. (1985) FEBS Lett. 182, 103–106.

[13] Declercq, J.P., Tinant, B., Parello, J., Etienne, G. and Huber, R. (1988) J. Mol. Biol. 202, 349–353.
[14] Swain, A.L., Kretsinger, R.H. and Amma, E.L. (1989) J. Mol. Biol. 264, 16620–16628.
[15] Kumer, V.D., Lee, L. and Edwards, B.F.P. (1990) Biochemistry 29, 1404–1412.
[16] Declercq, J.P., Tinant, B., Parello, J. and Rambaud, J. (1991) J. Mol. Biol. 220, 1017–1039.
[17] Padilla, A., Cavé, A. and Parello, J. (1988) J. Mol. Biol. 204, 995–1017.
[18] Padilla, A., Vuister, G.W., Boelens, R., Kleyvegt, G.J., Cavé, A., Parello, J. and Kaptein, R. (1990) J. Am. Chem. Soc. 112, 5024–5030.
[19] Lehky, P., Comte, M., Fischer, E.H. and Stein, E.A. (1977) Anal. Biochem. 82, 158–169.
[20] Wunk, W., Cox, J.A. and Stein, E.A. (1982) Calcium and Cell Function (Cheung, W.Y. ed.) vol. 2, pp. 243–278, Academic Press, New York.
[21] Tasumi, M. (1993) Fifth Internatioal Conference on the Spectroscopy of Biological Molecules (Theophanides, T., Anastassopoulou, J. and Fotopoulos, N. eds.) pp. 173–176, Kluwer, Dordrecht.
[22] Nara, M., Tanokura, M. and Tasumi, M. (1993) Proceedings of the 9th International Conference on Fourier Transform Spectroscopy, SPIE Vol. 2089 (Bertie, J.E. and Wieser, H. eds.) pp. 344–345.
[23] Pechère, J.F., Demaille, J.G. and Capony, J.P. (1971) Biochim. Biophys. Acta 236, 391–408.
[24] Yagi, K., Matsuda, S., Nagamoto, H., Mikuni, T. and Yazawa, M. (1982) Calmodulin and Intracellular Ca^{2+} Receptors (Kakiuchi, S. et al. eds.) pp. 75–91, Plenum, New York.
[25] Mantsch, H.H., Casal, H.L. and Mofatt, D.M. (1986) Spectroscopy of Biological Systems (Clark, R.J.H. and Hester, R.E. eds.) pp. 1–46, Wiley, Chichester.
[26] Surewicz, W.K. and Mantsch, H.H. (1988) Biochim. Biophys. Acta 952, 115–130.
[27] Arrondo, J.L.R., Muga, A., Castresana, J. and Goñi, F.M. (1993) Prog. Biophys. Mol. Biol. 59, 23–56.
[28] Jones, R.N. and Shimokoshi, K. (1983) Appl. Spectrosc. 37, 59–67.
[29] Lin-Vien, D., Colthup, N.B., Fateley, W.G. and Grasselli, J.G. (1991) The Handbook of Infrared and Raman Characteristic Frequencies of Organic Molecules, pp. 117–154, Academic Press, San Diego.
[30] Deacon, G.B. and Phillip, R.J. (1980) Coord. Chem. Rev. 33, 227–250.