Study of a murine IgG2a monoclonal antibody by vibrational spectroscopies

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The Raman and FTIR spectra of a murine IgG2a monoclonal antibody molecule in anhydrous state and in aqueous solutions of H_2O and D_2O are reported. The secondary structural characteristics have been investigated and have been established from absorption measurements in the amide I, I' and II, II' frequency range. In an anhydrous state, in accordance with previous studies on immunoglobulins, the secondary structures is predominantly of the β-sheet type. Data obtained in different polar media (KBr, H_2O, D_2O) reveal that IgG2a is a highly flexible protein. In pure H_2O and D_2O a rapid solvation of free peptide units occurs.

Keywords: Immunoglobulin; infrared absorption; Raman

Se presentan en este trabajo espectros Raman y FTIR de un anticuerpo monoclonal IgG2a en el estado anhidro y en soluciones de H_2O y D_2O. Las características de la estructura secundaria fueron investigadas y establecidas a partir de medidas de absorción en la región de las frecuencias de las vibraciones amida I, I' y II, II'. En el estado anhidro, de acuerdo con estudios anteriores sobre inmunoglobulinas, la estructura secundaria predominante es del tipo β-sheet. Los datos obtenidos en diferentes medios polares (KBr, H_2O, D_2O) revelan que la IgG2a es una proteína muy flexible. En H_2O y D_2O pura, ocurre una rápida solvatación de los grupos peptídicos libres.

Descriptores: Inmunoglobulina; absorción infraroja; Raman

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1. Introduction

A knowledge of protein conformation has been one of the major ambitions since the importance of a structure–function relationship was postulated. An investigation of the structure properties of protein should contribute to the understanding of their activities *in situ*. For many years now, Raman and infrared spectroscopies have been used to study the conformation of proteins in various forms. Conformational changes due to denaturation, chemical modifications, lyophilization and crystallization can be analyzed by these techniques. The structure of various immunoglobulins molecules and antibody-antigen precipitates has been analyzed in the mid seventies by Painter and Koenig [1] and later by Pezolet *et al.* [2]. The results indicate that the predominant structure in several immunoglobulins such as human IgG, IgM and rabbit IgG is the antiparallel β-sheet.

More recently the human monoclonal immunoglobulins; IgM-k McE, IgG-k Ger, IgM-k WSm and IgG-l Gui have also been analyzed by Raman spectroscopy. The obtained spectra show that all these immunoglobulins have similar secondary structures predominantly of the β-sheet type [3]. The various amide I, II and III modes corresponding to the secondary structure that a protein may adopt, *i.e.* α-helix, parallel and antiparallel β-pleated sheets, random coils, turns, and those resulting from the direct interactions between peptide units and solvents are largely overlapping. Moreover, amino acid side-chain absorptions may also contribute. Amide I, II and III have relatively strong infrared absorption. The amide II band is generally not observed in Raman scattering, or is very weak when it exists; in contrast, the amide I and III bands have strong Raman intensity [4].

Immunoglobulin G (or γ-globulin) IgG is the most important serum antibody (blood protein that plays an important role in the defense against infections). It recognizes and neutralizes foreign intruders like bacteria, viruses, and fungi. The immunoglobulin IgG2a is composed of two heavy (H) chains and two light (L) chains as seen on Fig. 1. The heavy chain consists of about 420 amino acid residues and the light chain about 210. These chains are linked by disulfide (–S–S–) bridges to from a somewhat flattened Y shape [5, 6]. It is these Y arms that attach to intruders, since antigen binding sites are situated on these arms. In the present work a murine IgG2a monoclonal antibody molecule has been investigated by Raman scattering and infrared absorption spectroscopies in the solid state and in aqueous solutions of H_2O and D_2O. We find similar results to those reported previously by other authors in closely related samples.

2. Experimental

The preparation of antibody is described in detail elsewhere [7]. IgG2a monoclonal antibody was obtained by fusion of immune spleen cells and HG-PRT myeloma cells from Balb/c mice. IgG2a samples for FTIR in solid state was made by mixing dialyzed and lyophilized powder in KBr pellets. For the Infrared study in the aqueous solutions the optical path length was of 7 mm for H_2O and 25 μm for D_2O. The spectra were recorded within 5 min after the addition of the neutral solvent and did not show any changes after this

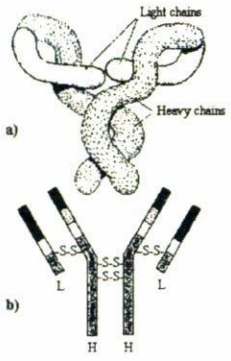

FIGURE 1. a) Highly simplified view of the immunoglobulin molecule IgG. Taken from [4]. b) Schematic representation of IgG. It is composed of 2 heavy (H) chains and 2 light (L) chains. These chains are linked disulfide bridge to form a Y shape. Taken from Ref. 7.

time. The spectra of the solvents were subtracted from the spectra of the solution in order to obtain a straight line from 1800 to 1200 cm^{-1}. FTIR spectra were recorded on a Nicolet 5DX FTIR spectrometer equipped with a globar source and a TGS (triglycine sulfate) detector. In order to eliminate the spectral contributions of the atmospheric water vapor and carbon dioxide, the instrument was purged with dry air. For each spectrum, 40 interferograms at 4 cm^{-1} resolution were collected, co-added, apodized with a Happ-Genzel function and Fourier transformed.

The various amide I, I' and II, II' modes corresponding to the major conformations that a protein may adopt are largely overlapping. Moreover amino-acid side-chain absorptions may also contribute to these bands. To process such complicated data the bands obtained between 1300–1800 cm^{-1} interval were decomposed directly in nine Lorentzian-Gaussians (with 70% Gaussian) by means of a least-squares fitting program (Axum 4.1 from Uniware) for the solid state; two adjustable parameters (intensity, bandwidth at half-height) were considered for each Lorentzian-Gaussian. For the solutions in water and deuterated water, the number of bands is eight and seven, respectively. In all cases, the frequency of the bands were taken from a second-derivative calculation of the spectrum. The initial intensities and bandwidth of each absorbance maximum of the original spectrum were chosen in order to give the best adjustment, and each assay was visualized before calculation was initialized. The percentages corresponding to the relative surface of each Lorentzian-Gaussian were determined in the two frequency ranges, 1600–1700 cm^{-1} and 1500–1600 cm^{-1}, attributed to the amide I, I' and II, $\nu_a COO^-$ regions respectively. The remaining components between 1500 and 1400 cm^{-1} were assigned to amide II', $\nu_s COO^-$ and CH deformation. The various structures displayed in the amide I, I' region are supposed to have close molar extinction coefficients [8], therefore their fractional areas are generally considered as representative of the relative proportions of the conformational structures adopted by a given protein [9, 10].

Raman spectroscopy measurements were performed on a Jobin Yvon Ramanor HG2S spectrometer. The excitation source was the 514.5 nm line of a 2017 Spectra Physics Argon ion laser. The irradiation intensity was of the order of 20 mW. All the experiments were performed at room temperature with dialyzed and lyophilized samples and aqueous solutions enclosed into sealed 1 mm diameter glass tubes. The scattering light, detected at right angle from the incident light, was collected on the photocathod of a cooled photomultiplier and amplified by a dc operational amplifier. It is important to note that during the Raman spectroscopy measurements the samples exhibited a strong luminescence which decays under laser irradiation. This effect has been described by Painter and Mosher [11]. The samples were thus left under irradiation for a few hours prior to start the Raman measurements.

3. Results and discussion

The effect of protein solvation prove to be rather complex. A close examination of the amide I, I' and II, II' modes reported in the literature and their correlation to a given structure or interactions appear of interest. Table Ia summarizes the different amide I, I' and amide II frequency ranges that have been associated with the well-known protein-secondary structures. The frequencies of the amide I, I' and II modes relevant to direct interactions between peptide units and solvents are reported in Table Ib. The frequencies observed in the same range for the amino acid side-chains are given in Table Ic [8–26].

The amide I vibrational mode is the in-plane peptide carbonyl stretching vibration (nearly 80%) [23] with a small contribution from the N–H in-plane bending vibration (10%) and C-N stretching (10%). From Table I, it has been assumed that absorption frequencies between 1660 and 1710 cm^{-1} are essentially related to non-hydrogen-bonded amide C=O groups involved in random coils, turns, and β-strands [24]. These C=O groups will be referred as "free" C=O groups. Absorptions between 1640 and 1670 cm^{-1} partially overlap the former frequency range. They are associated with hydrogen-bonded peptide units in α-helix, turns, and random coils [14, 18], but are also relevant to $C{=}O\cdots D_2O$ or $C{=}O\cdots Na^+$ interactions [20, 24]. Amide I frequencies in the 1626–1640 cm^{-1} range are attributed to parallel β-sheets [12, 13] but could as well be assigned to antiparallel β-strands (1612–1640 cm^{-1}) [19]. Finally, the amide I frequencies of antiparallel β-sheets of large size, which involves multistrands [18] as well as those associated with $C{=}O\cdots(D_2O)_2$ interactions [14, 18], are expected between 1610 and 1635 cm^{-1}. It has to be added that in recent works some features due to 310 helices appear near 1640 and 1662 cm^{-1} [25] and that a small absorption under 1640 cm^{-1} due to distorted helical structure was observed in some compounds with α-helix structure [26].

Increased hydrogen bonding decreases the frequency of the amide I band. When deuterated (amide I'), the hydrogen atom of the peptidic groups can be exchanged with a deuteri-

TABLA I. Frequency at maximum absorbance of the different amide band components in the 1700–1400 cm^{-1} frequency range.

	frequency (cm^{-1})		
	amide I	amide II	amide II'
(a) Protein secondary conformation			
α-helix	1657–1645	1550–1537 (strong)	1488
		1520–1510 (shoulder)	1464
antiparallel β-pleated sheet	1640–1612 (strong)	1530–1510 (strong)	1485–1464
	1699–1670 (weak)	1560–1555	
parallel β-pleated sheet	1642–1626	1553–1530	1480
random coil	1654–1640	1540–1530	
turns	1696–1681	1565–1530	
	1675–1655		
(b) Peptide unit-solvent interactions			
apolar solvent	1680–1665	1500 (medium)	1418
		1515 (shoulder)	1438
$C{=}O \cdots D_2O$	$\approx$1650		
$C{=}O \cdots (D_2O)_2$	$\approx$1620		
$C{=}O \cdots Na^+$	$\approx$1654		
$C{=}O \cdots (Na^+, D_2O)$	$\approx$1635		

		assignment	frequency (cm^{-1})
(c) Amino acid side-chain absorption			
Asp, Glu	COOD	νCO	1714, 1706
	COO^-	$\nu_a COO^-$	1584, 1567
		$\nu_s COO^-$	1405
Asp, Glu	$CO(ND_2)$	ν_nCO	1648, 1635
Arg	$D_2N{-}C{=}ND_2^+$	ν_a, ν_s N–C=N	1608, 1586
Tyr		ν skeleton	1515

um. This hydrogen/deuterium exchange should lead to a decrease of the amide I frequencies by about 5 cm^{-1} independently of any solvation or structural change compared to H_2O solution, in D_2O solution. A shift of the amide I band towards lower frequencies and exceeding 5 cm^{-1}, or towards higher frequencies will reflect in any case a solvent effect on the peptide groups. Amide I, I' bands can also overlapped bands relevant to direct interactions between side-chains and solvent (Table Ib).

The amide II mode does not involve the C=O vibration [23, 24], it is mainly composed of 40% C–N stretch and 60% N–H in-plane bend. This vibration is found between 1550 and 1537 cm^{-1} for α-helix conformation, between 1530 and 1510 cm^{-1} for antiparallel β-pleated sheet, between 1553 and 1530 cm^{-1} for parallel β-pleated sheet, between 1540 and 1530 cm^{-1} for random coils and between 1565 and 1530 cm^{-1} for turns. Increased hydrogen bonding on the N–H group, increases the frequency of the amide II band in contrast with the amide I band. The amide II band, in deuterated water, shows an intensity decrease and a concomitant intensity increase of a band between 1460 and 1480 cm^{-1} (amide II'). Contribution of ionized amino acid side-chains (Asp, Glu) may be significant in the amide II region especially when the intensity of this mode strongly decreases in D_2O solution (Table Ic).

The amide III is composed of 30% C–N stretch, and 40% N–H bend [28] and band is generally found between 1240 and 1300 cm^{-1}. It shows also a high frequency shift when the peptide bond C=O is involved in hydrogen bonding. The deuterium exchange reaction also leads to a significant isotope effect of the amide III band because its major contribution originates from the N–H bending.

In samples containing a carboxylic group (COOH), a strong feature is observed between 1650 and 1750 cm^{-1} due to the C=O stretching vibration mode. If a carboxylate COO^- group exists two strong bands are observed near 1600 and 1400 cm^{-1}, assigned to the antisymmetrical and the symmetrical COO^- stretching modes, respectively [4].

By Raman scattering [4], the amide I mode is generally found between 1645 and 1657 cm^{-1}, 1665 and 1680 cm^{-1}, 1662 and 1672 cm^{-1}, and, 1660 and 1665 cm^{-1} for α-helix, β-sheet, turns and random coils, respectively. The amide II

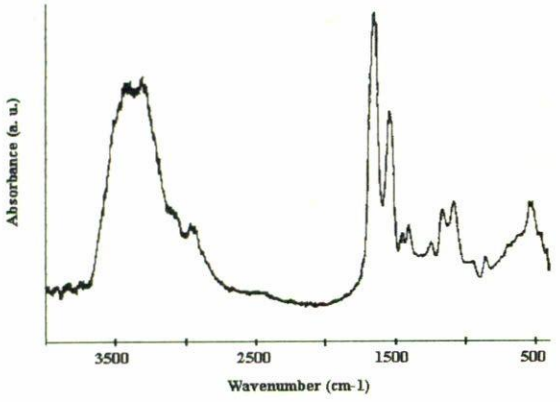

FIGURE 2. Infrared absorption spectrum of anhydrous IgG2a in KBr pellets at room temperature.

band is either not observed or very weak by Raman scattering, whereas the amide II' band stands at about 1470 cm^{-1} after deuteration. The amide III band is found for the same structures between: 1260 and 1305 cm^{-1}, 1230 and 1245 cm^{-1}, 1250 and 1330 cm^{-1}, and, 1240 and 1250 cm^{-1}, respectively. It is shifted to the 960–1000 cm^{-1} region after deuteration (amide III') [4, 28, 29].

These results will be presented hereafter, we will present first the protein in anhydrous state in KBr medium, then the protein solvated by H_2O and D_2O.

3.1. IgG2a in anhydrous state

3.1.1. Infrared absorption spectrum

The infrared spectrum of IgG2a in KBr pellet is presented in Fig. 2. It exhibits a very broad band at high frequency centered around 3350 cm^{-1} due to N–H and O–H stretching modes. At lower frequency, we find a broad amide I band centered at 1652 cm^{-1} with a bandwidth at half-height of $\approx$ 71 cm^{-1}, which suggests that various conformations are overlapping. The amide II band is found also as a broad band (69 cm^{-1}) at 1540 cm^{-1}. Other bands as the amide III and the S-S stretching modes are found at 1242 cm^{-1} and 534 cm^{-1}, respectively. All the bands and their assignment are presented in Table II.

After mathematical treatment in the 1300–1800 cm^{-1} frequency range (Fig. 3), *i.e.*, second derivative and fitting procedure, the four features of the amide I band were observed. Frequencies and percentages are given in Table III. These proportions are in good agreement with the results of Painter and Koenig [1] and Pezolet *et al.* [2] found in human IgG antibodies. The major feature was centered at ca. 1642 cm^{-1}. This frequency is too low to be associated with α-helix conformations but too high to be assigned to antiparallel β-pleated multistrand structures of large size. It might be rather associated with parallel β-pleated sheet conformations and/or antiparallel β-strands of small size and/or to random coil structures. According to the deconvoluted spectrum, the component around 1614 cm^{-1} could account for a weak

TABLA II. Infrared and Raman frequencies of IgG2a and their assignments in the solid state and in water and deuterated water at room temperature.

Solid		H_2O		D_2O		Assignment
IR	R	IR	R	IR	R	
534	505				502	S–S
	621					Phe (ring breathing)
	643					ν(C–S)+Tyr
	758				757	Trp
	829					Tyr
860	857					Tyr
	878					Trp
947	936		943		942	ν(C–C)
	959					-
					988	Amide III'
	1004		1003		1004	Phe
	1032		1032		1033	Phe
1098	1079				1098	ν(C–N)
	1125		1127			δ C–H (ring)
1160	1156		1157			-
	1174					Tyr,Phe
	1206		1209	1212	1213	Tyr, Phe
1242	1238	1243	1238		1234	Amide III
	1258		1332			Trp (indole ring)
	1314	1317	1332		1325	δ C–H
	1338				1340	Trp
1403	1396	1400	1392	1400		$\nu_s(COO^-)$
1447	1446		1449			δ CH2
	1457	1456				-
1540		1549		1455	1459	Amide II,II'
	1550	1565	1553	1564	1550	Trp
	1583			1580		$\nu_{as}(COO^-)$
	1604		1606			Tyr, Phe
	1616		1617			Tyr, Phe
1652	1669	1646	1666	1639	1661	Amide I. I'

amount of antiparallel β-sheets of large size. This may reflect that the amide I absorptions of antiparallel β-structures of large size are dispersed. The band at 1662.5 cm^{-1} could be assigned to hydrogen-bonded peptidic C=O groups involved in non-regular conformations, *i.e.* turns and random coils. The last band at ca. 1685 cm^{-1} should correspond to hydrogen-bonded involved in β-sheet structures but also to "free" peptidic C=O groups located in turns, unordered structures, and β-structures of small size.

The infrared amide II frequencies support the above conclusion, since parallel β-pleated sheets, antiparallel β-double strands, and turns could give rise to the two major features found at 1522.3 cm^{-1} (49.2%) and 1547.1 cm^{-1} (41.3%) whereas the small band at 1568.2 cm^{-1} (9.5%) could be rela-

TABLA III. Frequencies and fractional areas in the amide I,I' and the amide II, II bands of the IR spectrum of IgG2a.

medium	amide I,I' frequency (cm^{-1})				amide I, I' bandwidth at half-height (cm^{-1})	amide II,II' frequency (cm^{-1})			amide II,II' bandwidth at half-height (cm^{-1})
KBr	1614	1642	1662.5	1685	71	1522.3	1547.1	1568.2	69
	(31.3)	(31.1)	(25.9)	(11.7)		(49.2)	(41.3)	(9.5)	
H_2O		1643	1680	1693	98	1520	1549	1565	74
		(89)	(8)	(3)		(5)	(51.5)	(43.5)	
D_2O		1643	1680		50	1430	1457		72
		(92)	(8)			(81.5)	(18.5)		

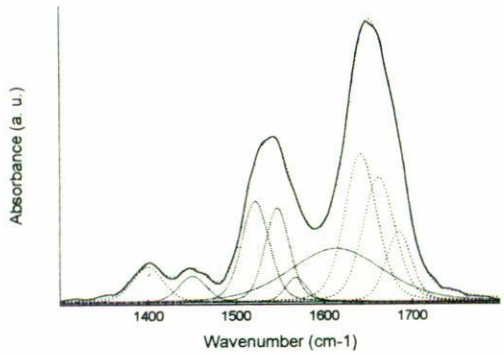

FIGURE 3. IR spectrum (full line) and decomposed spectrum (dotted line) of anhydrous IgG2a in KBr pellets at room temperature.

ted to the antisymmetrical stretching mode of COO^- groups of amino acid residues and correlated to the band at 1400 cm^{-1} which is the corresponding symmetrical mode [8, 25].

3.1.2. *Raman scattering spectrum*

In Fig. 4 the Raman spectrum of dialyzed and lyophilized IgG2a antibody is presented in the interval 900–1800 cm^{-1}. The principal peaks together with their assignment are shown in Table II. Strong bands corresponding to the amide I and amide III modes at 1668 cm^{-1} and 1238 cm^{-1}, respectively are clearly observable. The obtained spectrum is similar to the ones reported previously by other authors [1, 2]. The Amide II band is not observed on IgG2a. Raman spectroscopy provides additional evidence that IgG2a adopts mainly β-pleated sheet structures, since the maximum of the amide I peak is centered at 1668 cm^{-1}, and the amide III band at 1238 cm^{-1}.

Moreover, analysis of the spectrum between 600 and 900 cm^{-1} (spectrum not shown) can provide us more information about the position of some amino acid sidegroups [4]. The doublet at 857 and 829 cm^{-1} is characteristic of tyrosine fundamental vibration and overtone of a ring bending vibration. The intensity ratio I_{857}/I_{829} is found $\sim$ 1 that which means that 67% of the tyrosine residues are exposed and the

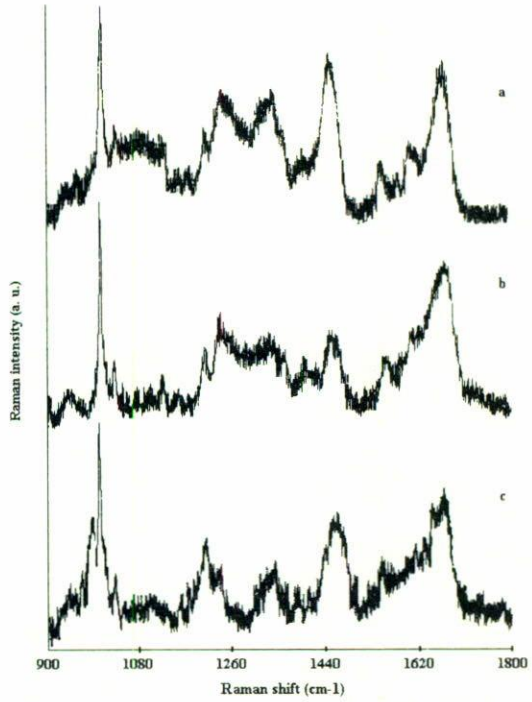

FIGURE 4. Raman spectrum of (a) anhydrous IgG2a powder at room temperature, (b) IgG2a solvated in H_2O at room temperature, (c) IgG2a solvated in D_2O at room temperature.

other ones are buried. An estimation of phenylalanine/tyrosine ratio can be obtained from the I_{621}/I_{643} intensity ratio. This ratio is $\sim$ 1.1 and we can estimate that there are 13 phenyalanine groups for 10 tyrosine groups. The fact that we did not observe an intense band near 1360 cm^{-1} seems to indicate that the tryptophan groups are exposed. The S–S bridge frequency is found at 505 cm^{-1} by Raman scattering. The discrepancy with the infrared absorption result is probably due to a centro-symmetric conformation of the –C–S–S–C– dihedral angle. At higher frequency, an intense band with many shoulders, due to N–H and O–H stretching modes, is observed. The O–H stretching bands are probably due to tyrosine groups and to some residual water molecules as well.

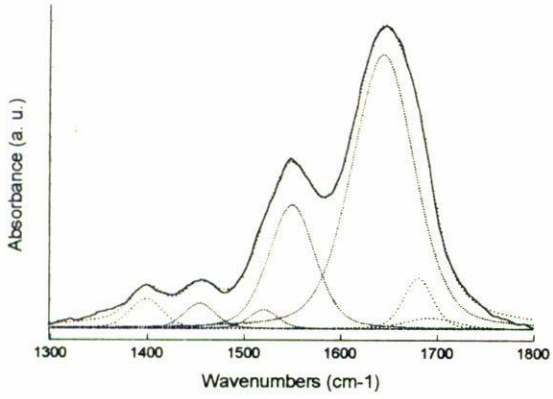

FIGURE 5. IR spectrum (full line) and decomposed spectrum (dotted line) of IgG2a solvated in H_2O at room temperature.

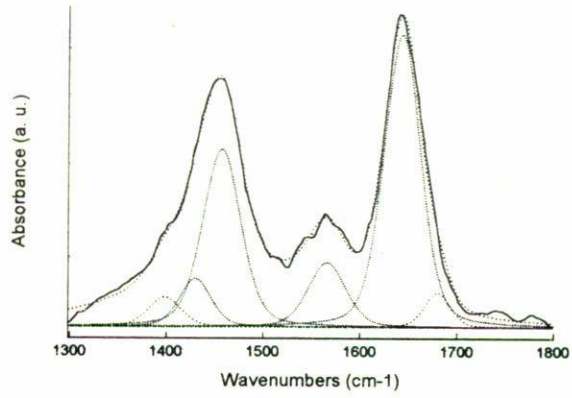

FIGURE 6. IR spectrum (full line) and decomposed spectrum (dotted line) of IgG2a solvated in D_2O at room temperature.

3.2. IgG2a in aqueous solutions

3.2.1. In water

The infrared absorption spectrum of IgG2a solvated in neutral solution of water at 25°C is shown in Fig. 5 in the amide I and II region where two maxima are observed at ca. 1646 and 1549 cm^{-1}. Others observed frequencies and tentative assignments are given in Table II. Compared to the anhydrous material, the amide I and II bands are broaden when IgG2a is solvated in water (98 and 74 cm^{-1}, respectively). This broadening suggests an enhancement of random structures. As we said above, with respect to the anhydrous state, solvation in water shifts the amide I band to lower frequency (-6 cm^{-1}) and the amide II band to higher frequency ($+9$ cm^{-1}). Nevertheless, presence of water absorption in the amide I region let us make difficult the analysis of this part of the spectrum.

After mathematical treatment of the amide I and II regions, three features are found in the amide I band at ca. 1643 cm^{-1} (89%), 1680 cm^{-1} (8%) and 1693 cm^{-1} (3%). Frequencies of the amide I and II bands and percentages are compared in Table III with those of the solid state. The main contribution corresponds to β-pleated sheet structures but is also due to water absorption. The small contribution at 1693 cm^{-1} in the amide I band corresponds to free C=O groups that are in smaller quantity than in the anhydrous state. That means that many of the free C=O groups of the solid state are now hydrogen bonded by water in solution. The amide II band has also three maxima (Table III) 1520 cm^{-1} (5%), 1549 cm^{-1} (51.5%) and 1565 cm^{-1} (43.5%). We can observe the important intensity decrease of the low frequency band, and consequently the increase of the two other bands. This indicates an increase of turns and random conformations and a decrease of the β-pleated sheet structures. This was also indicated by the disaparition of the low frequency band in amide I region.

By Raman scattering (Table II), the amide I band as a maximum at ca. 1666 cm^{-1} and the amide II band is not observed. The low frequency shift with respect to the anhydrous state (-3 cm^{-1}) of the amide I band indicates an increase of the hydrogen bonding of the C=O groups and confirms the analysis of the infrared results.

3.2.2. In deuterated water

The infrared absorption spectrum of IgG2a solvated in neutral solution of D_2O at 25°C exhibits three main peaks in the 1800-1300 cm^{-1} interval (Fig. 6) with maxima at 1639, 1564 and 1455 cm^{-1}. Substition of H_2O by D_2O shifts the water bending mode to lower frequency and thus clears the amide I, I' region which is sensitive to the conformation of the protein. Compared to solid state and to solvation in water, the above spectrum shows that the amide I' frequency slightly decreased (1639 cm^{-1}). The amide II' band (1455 cm^{-1}) is found at lower frequency due to the H–D exchange. The small band at ca. 1564 cm^{-1} has two shoulders at 1544 and 1580 cm^{-1}. The central band could be due to Trp residues. The high frequency shoulder to the antisymmetrical mode of COO^- groups which are more easily observed. The low frequency shoulder could be due to some peptidic groups very deeply buried in the protein and prevented from the H–D exchange and/or to δ CH_2 vibrations. The amide III' band is not observed by IR absorption due to the very important shift produced by the same phenomenon. Observed frequencies and tentative assignments are given in Table II.

After the different mathematical processings, the highest frequency component observed in the solid state has disappeared in D_2O (Fig. 6, Table III). This indicates that almost of the C=O groups, which were "free" in KBr, become deuterium or hydrogen bonded in D_2O. Consequently, either the percentage of β-structures increases (see Table Ia) or direct $C{=}O\cdots(D_2O)_{1-2}$ interactions are formed (see Table Ib) or both. Nevertheless, the absence of a low frequency band (near 1615-1620 cm^{-1}) in this region let us think that $C{=}O\cdots(D_2O)_2$ are very few. However, it could be assumed that the conformation of the protein is largely maintained in D_2O.

By Raman scattering (Table II), the amide I' is found at 1661 cm^{-1}, the amide II' at 1459 cm^{-1} and the amide III'

at 988 cm^{-1}. These frequencies confirms the above analysis, where the low frequency shift of the three bands result from the H–D exchange.

4. Conclusion

In this work a structural characterization of an IgG2a monoclonal antibody by Raman and Infrared spectroscopies confirms that the predominant structure is the antiparallel β-sheet. Analysis of the solvation of the protein by H_2O and D_2O was also established from the absorption measurements in the amide I, I' and II, II' frequency range. This analysis does not give absolute results, but as these experiments were held under the same conditions of protein concentration, pH, and temperature, this study is assumed to give a coherent description of the conformational dynamics of IgG2a when the protein is dissolved in various media. Hence, even weak variations observed from one spectrum to another have been reliably analyzed.

The highest amide I, I' component, the frequency and intensity of which depend on the solvent, can be mainly associated with "free" C=O groups, which are rapidly solvated by H_2O and D_2O. The occurrence of such "free" C=O groups must be correlated to "free" N–H groups which are rapidly exchanged in D_2O. From the occurrence of two components in the 1600–1645 cm^{-1} range two β-structures could be discriminated: antiparallel β-sheets of large size and parallel and/or antiparallel β-strands of smaller size. The large antiparallel β-structures give rise to the absorption observed at ca. 1614 cm^{-1} [8, 9]. The two others β-structures are indistinctly related to the component at ca. 1642 cm^{-1} [19].

The assignments proposed to analyze the solvation process of IgG2a emphasize the importance of peptide unit - solvent interactions. Analyses of the conformation in different aqueous media indicate that IgG2a is a flexible molecule. When IgG2a is dissolved in H_2O and D_2O, the "free" peptide units observed in the lyophilized form are rapidly solvated and exchanged.

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