Available online at www.sciencedirect.com
SCIENCE DIRECT®
Biochimica et Biophysica Acta 1614 (2003) 97–103
BIOCHIMICA ET BIOPHYSICA ACTA
BBA
www.bba-direct.com

Review

Attenuated total reflection IR spectroscopy as a tool to investigate the orientation and tertiary structure changes in fusion proteins

I. Martin, E. Goormaghtigh, J.-M. Ruysschaert*

Structure and Function of Biological Membranes, Center of Structural Biology and Bioinformatics, Université Libre de Bruxelles, Boulevard du Triomphe C.P. 206/2, B-1050 Brussels, Belgium

Received 28 April 2003; accepted 15 May 2003

Abstract

Membrane fusion proceeds via a merging of two lipid bilayers and a redistribution of aqueous contents and bilayer components. It involves transition states in which the phospholipids are not arranged in bilayers and in which the monolayers are highly curved. Such transition states are energetically unfavourable since biological membranes are submitted to strong repulsive hydration electrostatic and steric barriers. Viral membrane proteins can help to overcome these barriers. Viral proteins involved in membrane fusion are membrane associated and the presence of lipids restricts drastically the potential of methods (RMN, X-ray crystallography) that have been used successfully to determine the tertiary structure of soluble proteins. We describe here how IR spectroscopy allows to solve some of the problems related to the lipid environment.

The principles of the method, the experimental setup and the preparation of the samples are briefly described. A few examples illustrate how attenuated total reflection Fourier-transform IR (ATR-FTIR) spectroscopy can be used to gain information on the orientation and the accessibility to the water phase of the fusogenic domain of viral proteins. Recent developments suggest that the method could also be used to detect changes located in the membrane domains and to identify intermediate structural states involved in the fusion process.

Keywords: Attenuated total reflection; IR spectroscopy; Fusion peptide orientation; Hydrogen/deuterium exchange; Dichroic spectra

1. Introduction

The mechanism of membrane fusion involved in biological processes such as endo- or exocytosis, membrane recycling, fertilisation and enveloped virus infection is still unclear. The question arises whether these fusion reactions share features with each other. The best-characterised mechanisms of biological fusion are those used by enveloped virus, such as influenza virus, to enter their host cells. Membrane fusion proceeds via a merging of two lipid bilayers and a redistribution of aqueous contents and bilayer components. It involves transition states in which the phospholipids are not arranged in bilayers and in which the monolayers are highly curved [1–3]. Such transition states are energetically unfavourable since biological membranes are submitted to strong repulsive hydration electrostatic and steric barriers. These barriers can be overcome by membrane proteins. They facilitate local dehydration and are thought to induce local perturbations in the lipid bilayer through their insertion into membranes. Among the few well-characterised fusion proteins are viral spike glycoproteins responsible for penetration of enveloped virus into their host cells [4] and more particularly the hemagglutinin of influenza virus. In spite of similar functions, viral fusion proteins are diverse with little sequence similarities between various viruses [5–7]. In their sequences, these proteins posses a ‘‘fusion peptide’’, a short segment of relatively hydrophobic residue, commonly found in a membrane-anchored polypeptide chain [8]. Fusion peptide have also been identified in proteins involved in gamete fusion [9], in myoblast fusion [10], in vesicular fusion in neurons. Such peptides are implicated in the fusion process. In general, the exposed fusion peptides are believed to insert into the target cell membrane, and thus cause the local destabilisation of the lipid bilayer necessary to catalyse fusion. To simulate protein-mediated fusion, many studies on peptide-induced membrane fusion have been conducted on model membranes such as liposomes and have employed synthetic peptides corresponding to the putative fusion sequences of viral proteins [11–13].

* Corresponding author. Tel.: +32-2-650-53-77; fax: +32-2-650-53-77.
E-mail address: jmruyss@ulb.ac.be (J.-M. Ruysschaert).

0005-2736/03/$ - see front matter © 2003 Elsevier B.V. All rights reserved.
doi:10.1016/S0005-2736(03)00167-6

A molecular understanding of the mechanism by which these proteins induce fusion requires a deeper knowledge of the viral protein structure and of the structural states involved in the fusion process. Most of the methods that have been used successfully to determine the tertiary structure of soluble proteins cannot be used in a lipid environment and since most viral proteins are membrane proteins, a knowledge of their tertiary structure remains a major challenge, and explains that in most cases only the tertiary structure of ectodomains has been investigated as illustrated for ectodomain of the influenza HA protein for which atomic resolution is available for both its neutral and its low pH form [14,15].

X-ray crystallography provides accurate structural data, but obtaining high-quality crystals of membrane proteins is still extremely difficult. Reasonably high resolution structures have been obtained for only a few unrelated membrane proteins: [16–32]. The method is inherently static and the three-dimensional crystals necessary to obtain atomic resolution require the replacement of the lipid bilayer by detergents. NMR spectroscopy remains limited to small soluble proteins because the line-broadening effect associated with larger structures and nonisotropic motion of proteins within the lipid matrix. Large membrane vesicles containing both lipids and proteins or peptides are therefore still difficult to examine. CD suffers from light scattering and is prone to errors when large membrane fragments or lipid vesicles have to be studied. Under such conditions, a major effort is needed to correct the CD spectrum for flattening effects. Fluorescence spectroscopy often requires the use of perturbating probes and is also sensitive to light scattering effects. Application of Raman spectroscopy to the study of membrane proteins is promising, but is still limited by the low signal-to-noise ratio usually caused by interfering luminescence background with a strong contribution from chromophores. Raman spectroscopy does offer, however, a wealth of information on some amino acid side chains, which is complementary to the infrared data.

IR spectroscopy remains extremely useful as a tool to assess the structure of proteins that cannot be studied by X-ray crystallography and NMR. Attenuated total reflection Fourier-transform infrared spectroscopy (ATR-FTIR) is one of the most powerful methods for recording IR spectra of biological materials in general, and for biological membranes in particular. It is fast, yields a strong signal with only a few micrograms of sample, and most importantly provides information about the secondary structure, the orientation and the tertiary structure changes of a protein in an oriented system. The environment of the molecules can be modulated so that their conformation can be studied as a function of temperature, pressure and pH, as well as in the presence of specific ligands. Because of the long IR wavelength, light scattering problems are virtually nonexistent, and highly aggregated materials or large membrane fragments can be investigated. A unique advantage of IR spectroscopy is that it allows simultaneous study of the structure of lipids and proteins in intact biological membranes without introduction of foreign perturbing probes.

We describe here the principles of the method, the experimental setup and the preparation of the samples. A few examples illustrate how ATR-FTIR can be used to gain information on the orientation and the accessibility to the water-phase of the fusogenic domain of viral proteins. Recent developments suggest that the method could also be used to detect changes located in the membrane domains and to identify intermediate structural states involved in the fusion process.

2. Polarised ATR-FTIR

2.1. Experimental setup

Various experimental setups have been designed, including the use of optical fibres for the study of proteins [33–36]. However, the most usual design is the germanium trapezoidal plate, which allows molecular orientation to be determined by means of linear dichroism for oriented membranes. The IR beam is directed into a high refractive index medium that is transparent for the IR radiation of interest. Above a critical angle, which depends on the refractive index of the plate and of the external medium, the light beam is completely reflected when it impinges on the surface of the plate. Several internal reflections occur within the plate until the beam reaches the end. A detailed description of the setup is available in Ref. [37]. Samples that are deposited on the germanium plate absorb electromagnetic radiation of the evanescent wave and thereby reduce the intensity of the reflected light. Hence, the technique is referred to as ‘‘attenuated total reflection Fourier-transform IR spectroscopy’’.

2.2. Sample preparation

The sample preparation for ATR-FTIR spectroscopy consists in spreading a drop of the sample, typically 5–50 μl containing 1–200 μg of either a membrane suspension or a protein solution on the germanium plate. The solvent is slowly evaporated under a gentle N2 flow while a Teflon bar or pipette tip is used to spread the liquid over the useful surface in order to make the film as uniform as possible. While evaporating, capillary forces flatten the membranes, which spontaneously form oriented multilayer arrangements [38]. The technique is very convenient for studying proteins inserted into reconstituted vesicles (liposomes, virosomes) can be used.

The possibility to gain information on the orientation of different regions of the phospholipid molecules and on the different protein secondary structures is certainly one of the most exciting possibilities of ATR-FTIR spectroscopy. A proper interpretation of the results supposes a minimum understanding of the principles of the method.

2.3. General principles

The method is based on the fact that the IR light absorption is maximal if the dipole transition moment is parallel to the electric field component of the incident light.

$$A\alpha\left|\frac{\partial\bar{\mu}}{\partial Q}\bar{E}\right|^2=\left|\frac{\partial\bar{\mu}}{\partial Q}\right|^2\left|\bar{E}\right|^2\cos^2\Gamma \quad (1)$$

where Γ is the angle between $((\partial\bar{\mu})/(\partial Q))$ and $\bar{E}$.

In an ordered membrane deposited on the germanium crystal, all the molecules, and therefore the transition dipole moments within the membrane molecules, have the same orientation with respect to a normal to the IRE surface. By measuring the spectral intensity while turning the incident light electric field orientation with a polariser, it is possible to gain information on the orientation of the dipoles. In fact, all the orientational information is contained in the dichroic ratio R^{ATR}, which is the ratio of the integrated absorbance of a band measured with a parallel polarisation of the incident light $A_{\parallel}$ to the absorbance measured with a perpendicular polarisation of the incident light $A_{\perp}$:

$$R^{\mathrm{ATR}}=\frac{A_{\parallel}}{A_{\perp}} \quad (2)$$

R^{ATR} can be computed by averaging for all orientations around the germanium crystal as well as for all orientations with respect to this normal. The former averaging is trivial since we suppose here a uniaxial symmetry for the distribution around this normal (for details of the derivation, see Ref. [39]). The latter averaging must take into account the distribution of the orientations with respect to the normal described by density distribution function. The dichroic ratio R^{ATR} is related to an orientational order parameter

$$R^{\mathrm{ATR}}=\frac{E_x^2}{E_y^2}+\frac{E_z^2}{E_y^2}\left(1+\frac{3S}{1-S}\right) \quad (3)$$

where E_x^2, E_y^2 and E_z^2 are the time-averaged square electric field amplitudes of the evanescent wave in the film at the plate/film interface already described in Ref. [37]. Interestingly, the evanescent wave exhibits electric field components in all directions. Therefore, at the opposite to the transmission situation, no tilt of the oriented membrane is necessary to gain full information on dipole orientation. S is defined as the projection of the density distribution function $C(\Gamma)$ on the second Legendre polynomial. For uniaxial symmetry, it is computed as

$$S=\int_0^{\pi}\frac{3\cos^2\Gamma-1}{2}C(\Gamma)\sin(\Gamma)\mathrm{d}\Gamma \quad (4)$$

where Γ refers to the considered distribution. It appears here that if the angular distribution function is developed in Legendre polynomials, only the second term of this development governs the dichroism in ATR-FTIR. The reader is referred to Ref. [37] for derivation of these equations for the specific case of ATR. For clarity of the subsequent discussion, we consider below that the orientation of an α-helix long axis with respect to the plate surface normal must be determined. The measured order parameter S, denoted below $S_{\mathrm{experimental}}$, obtained from R^{ATR} through Eq. (2), can be generally expressed as a set of uniaxial symmetric distributions:

$$S_{\mathrm{experimental}}=S_{\mathrm{membrane}}\cdot S_{\mathrm{helix}}\cdot S_{\mathrm{dipole}} \quad (5)$$

where S_{membrane} describes the distribution function of the lipid membrane patches (smallest planar membrane unit) with respect to the internal reflection element, S_{helix} describes the orientation of the helices with respect to the membrane plane, and S_{dipole} describes the dipole orientation of either amide I or amide II with respect to the helix axis. S_{helix} can be further split by describing it as the convolution of an order function $S_{\mathrm{helix\ order}}$ by a delta function describing the mean angle of the helix with respect to a membrane perpendicular $S_{\mathrm{helix\ angle}}$:

$$S_{\mathrm{helix}}=S_{\mathrm{helix\ angle}}\cdot S_{\mathrm{helix\ order}} \quad (6)$$

Because the transition dipole has a unique distribution of the angle α, it can generally be described by a delta function. Eq. (4) becomes

$$S_{\mathrm{dipole}}=\frac{3\cos^2\alpha-1}{2} \quad (7)$$

$S_{\mathrm{helix\ angle}}$ can now be evaluated from $S_{\mathrm{experimental}}$. Assuming $S_{\mathrm{helix\ order}}=1$, the tilt β of the helix axis with respect to the membrane normal can be determined provided that S_{dipole} and S_{membrane} are known. It is usually considered that $S_{\mathrm{membrane}}=1$ and S_{dipole} is a characteristic of the secondary structure:

$$\beta=\arccos\frac{2.S_{\mathrm{helix\ angle}}+1}{3} \quad (8)$$

2.4. Orientation of fusion peptides

A peptide can adopt defined orientation with respect to the lipid matrix. We illustrate here with a few examples how such an orientation can be determined by ATR-IR spectroscopy. For amphipathic peptides, it is the distribution of amino acids on the helix surface that determines the position in the lipid bilayer. This point has been illustrated with LAH_4, a synthetic amphipathic helical peptide that has been designed to change its orientation in the membrane in a pH-dependent manner and allows the evaluation of the hydrophobic, polar and electrostatic contributions that determine the orientation of membranes polypeptides. LAH_4 is 20 amino acids long and contains 4 histidines; the other amino acids (leucine, alanine) contribute to its hydrophobic character. The histidines exhibit pK values between 5.4 and 6.0 and the electrostatic contributions during membrane insertion are therefore strongly dependent on pH. LAH_4 was inserted into

lipid vesicles and polarised IR spectra revealed unambiguously that at low pH, the helix axis is oriented close to the membrane plane. At high pH, the helix axis is parallel to the lipid acyl chains [40,41]. This peptide orientation has been characterised by multidimensional solution and proton-decoupled ^{15}N solid state NMR spectroscopy [42].

A new factor that has emerged from the ATR-IR studies was that HIV/SIV peptides were obliquely oriented into the lipid bilayer of the host cell [43–45]. This was originally predicted by calculation of the directions of the hydrophobic moments, assuming that the sequences adopted a helical conformation. It has been hypothesised that such an orientation requires the existence of a gradient of hydrophobicity and hydrophilicity along the helix axes. Similar oblique insertion angles were found for the HA fusion peptide when using the same experimental conditions [46]. Mutation studies to deliberately alter the oblique angle of insertion were found to disrupt fusion efficiency in both intact virus [47,48] and synthetic fusion peptide systems [44,45,49,50] utilising HIV/SIV and Bovine Leukemia Virus. Small-angle neutron scattering from oriented membranes have also suggested that SIV viral fusion peptide containing substituted deuterium atoms is inserted into the lipid bilayer with an oblique angle [51]. Recent NMR and spin label quenching data suggest that a kink in the fusion peptide modifies the mode of insertion of the peptide in the bilayer [52,53]. Similar insertion modes have not been identified for other types of small membrane-associating peptides and may be specific to the fusogenic peptides. Support for the importance of the orientation also comes from studies in which the N-terminal of the fusion peptides is labelled with the NBD fluorophore, the emission properties of, which are sensitive to the polarity of the environment. For the Sendai fusion peptide, the N-terminal of the more fusogenic G12A mutant was found to be closer to the surface of the membrane than the wild-type, thus indicating a more oblique (closer to parallel) angle of insertion [54]. Fluorescently labelled peptide analogues can be used to determine the peptide–membrane partition coefficient and the position of the N terminus or C terminus in the membrane. Using this technique for the fusion protein of Sendai virus [54], show that a 33-residue peptide corresponding to the NH_2 terminus of the wild-type fusion protein is located within the lipid bilayer and that the peptide tends to self-associate in the membrane-bound state, while an ''nonfusogenic'' mutant peptide lies on the surface of the bilayer but has the same tendency to self-associate. The observed differences in the peptide fusogenic activities are hypothesised to result from the difference in the degree of penetration of the peptide into the membrane [54]. Similar results were found for the HIV fusion peptide, in which the N-terminal of the inactive V2E mutant was inserted deeper than the wild type [50]. In addition, both the membrane-bound Sendai and HIV fusion peptides were found to be efficiently cleaved by proteinase K, thus indicating only a shallow penetration into the bilayer. This is in contrast to transmembrane-bound peptides, which are typically protected from enzymatic cleavage [55]. Additional studies indicate that the oblique angle of the inserted helix causes a significant disruption of the alignment of the phospholipid alkyl chains, which does not occur for the parallel or perpendicular orientations [56,57]. It is postulated that this gives rise to the lipid phases that are associated with the initial events of membrane fusion. Since the oblique orientation prevents the helix from fully transversing the bilayer, it has also been suggested that this causes an expansion of the membrane core, which in turn increases negative curvature strain, destabilises the bilayer relative to the formation of inverted phases and facilitates membrane fusion [49]. The negative curvature strain would probably facilitate contact between the viral and target cell membranes, which would also probably increase the number of fusion proteins inserted simultaneously into both membranes. In addition, the resultant invagination will produce a region of high curvature at which membrane fusion is likely to commence [58].

Fertilin is a guinea pig sperm surface membrane protein that seems to play a role in sperm–egg membrane fusion. The two subunits α and β of fertilin share membrane topologies and other characteristics with viral binding and fusion proteins [59,60]. The β subunit contains a potential receptor binding domain and the α subunit contains a putative fusion peptide [9,10,61], suggesting that fusion events which occur during fertilisation may share a common mechanism with penetration of enveloped viruses into host cells. The sequence recently suggested to be involved in fusion comprises residues 89–111 of the α-subunit of fertilin [9]. This sequence fulfils all three criteria of an internal fusion peptide: (a) located in a membrane anchored subunit, (b) relatively hydrophobic and (c) able to be modelled as a ''sided'' α-helix with most of the bulky hydrophobic residues on one face and charged amino acids on the other face [10]. This peptide has been shown to cause membrane lysis and fusion [62].

The ATR-FTIR study [63] supports a model where the peptide, upon binding to the lipid phase, adopts an almost helical conformation with unordered structures only at the ends of the helix. The contribution of the β-structure was weak, suggesting that the β-sheet is not the main structure of the membrane-bound fertilin peptide. There appears to be a strong correlation between the oblique insertion of fertilin fusion peptide into the membrane, its fusogenic properties and its α-helical structure as shown for the viral fusion peptide [63].

In summary, the position and orientation of the viral fusion peptide within the fusion site is at least as important as its hydrophobicity and secondary structure.

3. Amide hydrogen/deuterium (H/D) exchange kinetics

Hydrogen isotope exchange has long been used for the analysis of protein structure and dynamics [64–66] (for a review, see Refs. [67,68]). It appeared to be one of the main techniques able to identify submolecular motional domains including fast exchanging protons of the protein surface,

somewhat slower exchanging protons of the flexible (loop) regions buried in the protein or involved in some secondary structures and the slowly exchanging protons from the protein core formed by the most rigid clusters (knots) of amino acids (for a review, see Ref. [69]). It has been suggested by Lumry [69] that the description of proteins in terms of three types of regions characterised by their particular dynamic structure is functionally and evolutionarily more relevant than a description in terms of secondary structures. The slowly exchanging cluster has been related for several proteins to the initial folding core during the sequence of events leading to protein folding [70]. One particularity of IR spectroscopy is that it allows one to monitor the exchange rate of the amide protons only, yielding data that are directly proportional to the number of amino acid residues in the protein. Recent developments indicate that a careful analysis of amide I in the course of deuteration makes it possible to assign the exchanging protons to a secondary structure type [71,72]. The high quality of the spectra obtained by the ATR-FTIR method should allow further developments of its use in this domain. The main advantage of this technique is that it provides information about tertiary structural modifications resulting from an environmental change (pH, ligand) even though the secondary structure is not altered.

3.1. Experimental procedure and data recording

Since the recording of H/D exchange kinetics by ATR-FTIR has only begun [73–80] but is not yet a standard procedure, we briefly describe here the experimental procedure. Thin films are obtained by slowly evaporating a sample containing typically 100 μg of protein or membrane suspension on one side of the ATR plate under a stream of nitrogen. However, much smaller amounts can be used. Successful H/D exchange analyses of reconstituted proteins, like P-glycoprotein [80] or LmrA [73], were performed on 20 μg of protein per experiment. The germanium crystal is then placed in an ATR holder for liquid sample with an inlet and outlet. H/D exchange is obtained by flushing the sealed chamber in contact with the sample with nitrogen gas saturated with D_2O by bubbling through a series of four vials containing D_2O at a flow rate of 50 ml min^{-1}, controlled by a flow meter. Amide H/D exchange was measured by monitoring the decrease in the amide II absorption (1544 cm^{-1}) as a function of the time of exposure to D_2O-saturated N_2. Under the conditions described above, the availability of D_2O is not rate limiting for the exchange. Moreover, varying the amount of material (from 10 to 150 μg) spread on the germanium crystal has no influence on the kinetics measured, indicating that the thickness of the film does not influence the D_2O diffusion in the film.

3.2. Amide H/D exchange in fusion peptides

The kinetics of deuteration of the fertilin fusion peptide associated to LUV PC/PS (80:20%) and LUV PC/PS (60:40%) were measured [63], at constant pH (7.2) and temperature (20 °C). The rate of amide H/D exchange is related to the stability of the secondary structure and to the solvent accessibility of the NH amide group of the protein or peptide. Amide hydrogen exchange was measured by monitoring the decrease in the amide II absorption (maximum at 1544 cm^{-1}) as a function of the time of exposure to D_2O-saturated N_2 (from 15 s to 2 h). The percentage of deuteration of fertilin peptide associated to LUV of PC/PS (80:20%) and PC/PS (60:40%) was calculated from the amide II/amide I ratio. H/D exchange was faster and almost complete for the peptide associated to the LUV containing 20% negatively charged lipids. After 20-min deuteration, 90% of the peptide N–H groups were exchanged, suggesting a complete accessibility of the peptide to the solvent, while when the fertilin peptide was associated to vesicles containing 40% PS, only 20% of the peptide NH groups were exchanged, suggesting less accessibility of the peptide to the solvent [63]. This result fully confirms the FTIR orientation, i.e., for vesicles containing 20% PS, the helix is parallel to the lipid membrane and accessible to the solvent, while for vesicle containing 40% PS, the helix is inserted obliquely in the lipid bilayer and much less accessible to the solvent.

A simplified membrane system, consisting of a fusion peptide anchored to donor vesicles and a population of target vesicles [81], demonstrated that fusion can be regulated by a switch of the secondary structure of the peptide from a nonfusogenic β-sheet to a fusion-permissive α-helix, and vice versa. The positioning of the switch is governed by the presence of distinct lipids in the target membrane [81]. The accessibility to the peptide to deuterium oxide was lower in the presence than in the absence of target membranes confirming that in these conditions the peptide was shielded from the aqueous environment. Surprisingly, at condition at which fusion is inhibited, i.e., in the presence of lysophosphatidylcholine (LPC), a further decrease in the accessibility of the peptide to D_2O was observed. Prior to fusion, the peptide was fully accessible to H/D exchange, which was completed after approximately 10–15 min. After fusion with target vesicles in the absence of LPC, this exchange amounted to approximately 50–60%. The exchange was severely diminished when the target contained LPC [81]. The degree of exchange is such (approximately 20% compared with approximately 50% at control condition) that it is unlikely that the exchange data can be explained by an enhanced insertion of the peptide at nonfusogenic conditions. Rather, a reasonable explanation could be that the peptide becomes clustered at the lipid/water interface in the β-sheet structure.

4. Perspectives

It is obvious that studies with isolated peptides and liposomes should be interpreted with care. A major point

concerns the fact that the structure of the peptides could be different from the one they adopt in the intact molecule.

A new approach has been recently developed in our group [82,83] in order to provide information about membrane domains dynamics. Monitoring the IR linear dichroism spectra in the course of H/D exchange allowed to focus the recording of exchange rates on the membrane-embedded regions of the protein only. The dichroism present in the intact protein can be completely explained by the dichroism of the helices, which remain associated with the membrane after proteinase K treatment [82,83]. This observation strongly suggests that only the transmembrane helices contribute significantly to the dichroism. Therefore this makes possible to monitor structural changes occurring in the membrane domains while working with the intact proteins. The analysis of the structure and orientation of the fusion peptide in viral proteins could be the benefit of this new approach.

In most cases, viral envelope proteins must first be activated by a specific environmental stimulus before fusion can start [84]. Such processing results in the exposure of the fusion peptide at this N-terminal position. For the well-studied hemagglutinin protein (HA) from influenza virus, activation occurs by the exposure to an acidic environment in the endosome vesicle (ca. pH 5.0–6.0), whereas for gp120/41 (*env*) of HIV, activation occurs by binding with receptor molecules in the target cell membrane (i.e., CD4 and CXCR4 or CCR5) [85–89]. In most of these systems, the structural changes induced by a change in the environmental conditions are limited to a small fraction of amino acids. The high sensitivity of the H/D exchange measurements allows to detect structural changes limited to a few amino acids and would be an elegant way to characterise in viral proteins structural changes due to environmental modifications.

The fusion peptides are much less fusogenic than the corresponding intact fusion protein. The factors explaining this discrepancy have been discussed extensively (multimers formation, role of other domains of the protein). Clearly, the mechanism by which viral proteins facilitate the formation of fusion intermediates is a complex process involving several domains of the fusion protein. Combining fluorescence quenching experiments and ATR-IR spectroscopy is a possible approach to learn more about these intermediate structural states and to study how such structural changes are propagating along the protein structure [83].

IR dichroic spectra reflect conformational changes occurring in the transmembrane, whereas fluorescence quenching of tryptophan residues by soluble attenuators reveals structural changes located in the cytosolic domains. This new approach has allowed to characterise structural states implicated in drug transport mediated by resistance proteins [82,83].

The fact that new IR developments allows to focus specifically on structural changes occurring in the membrane or in the soluble domain of a membrane protein and that more purified viral proteins are available makes this experimental strategy realistic and promising.

Acknowledgements

Erik Goormaghtigh is a Research Director at F.N.R.S. (Fonds National de la Recherche Scientifique).

This work was supported by F.N.R.S. and A.R.C. (Actions de Recherches Concertées).

References

[1] K.N.J. Burger, in: R.M. Epand (Ed.), Current Topics in Membranes, vol. 44, Academic Press, San Diego, 1998, pp. 403–445.
[2] L.V. Chernomordik, J. Zimmerberg, Curr. Opin. Struct. Biol. 5 (1995) 1–7.
[3] R.M. Epand, Biochem. Cell. Biol. 68 (1990) 17–23.
[4] M. Marsh, A. Helenius, Adv. Virus Res. 36 (1989) 107–151.
[5] W.R. Gallaher, J.M. Ball, R.F. Garry, M.C. Griffin, R.C. Montelaro, AIDS Res. Hum. Retrovir. 5 (1989) 431–440.
[6] M.J. Gething, R.W. Doms, D. York, J. White, J. Cell Biol. 102 (1986) 11–23.
[7] F. Gonzalez-Scarano, M.N. Waxham, A.M. Ross, J.A. Hoxie, AIDS Res. Hum. Retrovir. 3 (1987) 245–252.
[8] M.L. Bosch, P.L. Earl, K. Fargnoli, S. Picciafuoco, F. Giombini, F. Wong-Staal, G. Franchini, Science 244 (1989) 694–697.
[9] C.P. Blobel, T.G. Wolfsberg, C. Turck, D.G. Myles, P. Primakoff, J.M. White, Nature 356 (1992) 248–252.
[10] J.M. White, Science 258 (1992) 917–924.
[11] S.R. Durell, I. Martin, J.-M. Ruysschaert, Y. Shai, R. Blumenthal, Mol. Membr. Biol. 14 (1997) 97–112.
[12] I. Martin, E.I. Pécheur, J.-M. Ruysschaert, D. Hoekstra, Biochemistry 38 (1999) 9337–9347.
[13] E.I. Pécheur, J. Sainte-Marie, A. Bienvenüe, D. Hoekstra, J. Membr. Biol. 167 (1999) 1–17.
[14] I.A. Wilson, J.J. Skehel, D.C. Wiley, Nature 289 (1981) 366–373.
[15] P.A. Bullough, F.M. Hughson, J.J. Skehel, D.C. Wiley, Nature 371 (1994) 37–43.
[16] J. Deisenhofer, O. Epp, K. Miki, R. Huber, H. Michel, Nature 318 (1995) 618–624.
[17] H. Michel, J. Mol. Biol. 158 (1982) 567–572.
[18] J. Koepke, X. Hu, C. Muenke, K. Schulten, H. Michel, Structure 4 (1996) 581–597.
[19] N.W. Isaacs, R.J. Cogdell, A.A. Freer, S.M. Prince, Curr. Opin. Struct. Biol. 5 (1995) 794–797.
[20] W. Kuhlbrandt, D.N. Xang, Y. Fujiyoshi, Nature 367 (1994) 614–621.
[21] R. Henderson, P.N. Unwin, Proc. Natl. Acad. Sci. U. S. A. 257 (1975) 28–32.
[22] R. Henderson, J.M. Baldwin, T.A. Ceska, F. Zemlin, E. Beckmann, K.H. Downing, J. Mol. Biol. 213 (1990) 899–929.
[23] M.S. Weiss, A. Kreusch, E. Schiltz, U. Nestel, W. Welte, J. Weckesser, G.E. Schulz, FEBS Lett. 280 (1991) 379–382.
[24] D. Picot, P.J. Loll, R.M. Garavito, Nature 367 (1994) 243–249.
[25] C. Ostermeier, A. Harrenga, U. Ermler, H. Michel, Proc. Natl. Acad. Sci. U. S. A. 94 (1997) 10547–10553.
[26] T. Tsukihara, H. Aoyama, E. Yamashita, T. Tomizaki, H. Yamaguchi, I.K. Shinzawa, R. Nakashima, R. Yaono, S. Yoshikawa, Science 269 (1995) 1069–1074.
[27] T. Tsukihara, H. Aoyama, E. Yamashita, T. Tomizaki, H. Yamaguchi, I.K. Shinzawa, R. Nakashima, R. Yaono, S. Yoshikawa, Science 272 (1996) 1136–1144.
[28] I.K. Shinzawa, H. Ueda, S. Yoshikawa, H. Aoyama, E. Yamashita, T. Tsukihara, J. Mol. Biol. 246 (1995) 572–575.
[29] C. Toyoshima, M. Nakasako, H. Nomura, H. Ogawa, Nature 405 (2000) 647–655.
[30] K.R. MacKenzie, J.H. Prestegard, D.M. Engelman, Science 276 (1997) 131–133.

[31] I.T. Arkin, P.D. Adams, A.T. Brunger, S.O. Smith, D.M. Engelman, Annu. Rev. Biophys. Biomol. Struct. 26 (1997) 157–179.
[32] I.T. Arkin, P.D. Adams, K.R. MacKenzie, M.A. Lemmon, A.T. Brunger, D.M. Engelman, EMBO J. 13 (1994) 4757–4764.
[33] S. Simhony, A. Katzir, E.M. Kosower, Anal. Chem. 60 (1988) 1908–1910.
[34] S. Simhony, I. Schnitzer, A. Katzir, E.M. Kosower, J. Appl. Phys. 64 (1988) 3732–3734.
[35] S. Simhony, E.M. Kosower, A. Katzir, Biochem. Biophys. Res. Commun. 142 (1987) 1059–1063.
[36] S. Simhony, E.M. Kosower, A. Katzir, Appl. Phys. Lett. 49 (1986) 253–254.
[37] E. Goormaghtigh, V. Raussens, J.-M. Ruysschaert, Biochim. Biophys. Acta, Rev. Biomembr. 1422 (1999) 105–185.
[38] U.P. Fringeli, H.H. Gunthard, Mol. Biol. Biochem. Biophys. 31 (1981) 270–332.
[39] E. Goormaghtigh, J.-M. Ruysschaert, in: R. Brasseur (Ed.), Molecular Description of Biological Membranes by Computer Aided Conformational Analysis, CRC Press, Boca Raton, FL, 1990, pp. 285–329.
[40] E. Goormaghtigh, V. Cabiaux, J.-M. Ruysschaert, Subcell. Biochem. 23 (1994) 405–450.
[41] B. Bechinger, J.-M. Ruysschaert, E. Goormaghtigh, Biophys. J. 76 (1999) 552–563.
[42] B. Bechinger, J. Mol. Biol. 263 (1996) 768–775.
[43] I. Martin, F. Defrise-Quertain, E. Decroly, M. Vandenbranden, R. Brasseur, J.-M. Ruysschaert, Biochim. Biophys. Acta 1145 (1993) 124–133.
[44] I. Martin, M.C. Dubois, F. Defrise-Quertain, T. Saermark, A. Burny, R. Brasseur, J.-M. Ruysschaert, J. Virol. 68 (1994) 1139–1148.
[45] I. Martin, H. Schaal, A. Scheid, J.-M. Ruysschaert, J. Virol. 70 (1996) 298–304.
[46] J. Lüneberg, I. Martin, F. Nussler, J.-M. Ruysschaert, A. Herrmann, J. Biol. Chem. 270 (1995) 27606–27614.
[47] M. Horth, B. Lambrecht, C. Marinee, F. Bex, C. Thiriart, J.-M. Ruysschaert, A. Burny, R. Brasseur, EMBO J. 10 (1991) 2747–2755.
[48] V. Vonèche, D. Portetelle, R. Kettman, L. Willems, K. Limbach, E. Paoletti, J.-M. Ruysschaert, A. Burny, R. Brasseur, Proc. Natl. Acad. Sci. U. S. A. 89 (1992) 3810–3814.
[49] A. Colotto, I. Martin, J.-M. Ruysschaert, A. Sen, S.W. Hui, R.M. Epand, Biochemistry 35 (1996) 980–989.
[50] Y. Kliger, A. Aharoni, D. Rapaport, P. Jones, R. Blumenthal, Y. Shai, J. Biol. Chem. 272 (1997) 13496–13505.
[51] J.P. Bradshaw, M.J. Darkes, T.A. Harroun, J. Katsaras, R.M. Epand, Biochemistry 39 (2000) 6581–6585.
[52] X. Han, J.H. Bushweller, D.S. Cafiso, L.K. Tamm, Nat. Struct. Biol. 8 (2001) 715–720.
[53] H.J. Chu, J.W. Shih, K.C. Ding, C. Chinpan, J. Biol. Chem. 277 (2002) 22725–22733.
[54] D. Rapaport, Y. Shai, J. Biol. Chem. 269 (1994) 15124–15131.
[55] E. Gazit, Y. Shai, Biochemistry 32 (1993) 12363–12371.
[56] R.M. Epand, R.F. Epand, Biochem. Biophys. Res. Commun. 202 (1994) 1420–1425.
[57] R.F. Epand, I. Martin, J.-M. Ruysschaert, R.M. Epand, Biochem. Biophys. Res. Commun. 205 (1994) 1938–1943.
[58] A.M. Haywood, B.P. Boyer, Biochim. Biophys. Acta 646 (1981) 31–35.
[59] P. Primakoff, D.G. Hyatt, J. Tredick Kline, J. Cell Biol. 104 (1987) 141–149.
[60] C.P. Blobel, D.G. Myles, P. Primakoff, J. White, J. Cell Biol. (1990) 67–78.
[61] T. Wolfsberg, F. Bazan, C. Blobel, D. Myles, P. Primakoff, J. White, Proc. Natl. Acad. Sci. U. S. A. 90 (1993) 10783–10787.
[62] I. Martin, J.-M. Ruysschaert, FEBS Lett. 405 (1997) 351–355.
[63] I. Martin, R.M. Epand, J.-M. Ruysschaert, Biochemistry 37 (1998) 17030–17039.
[64] R.B. Gregory, R. Lumry, Biopolymers 24 (1985) 301–326.
[65] D.G. Knox, A. Rosenberg, Biopolymers 19 (1980) 1049–1068.
[66] S.W. Provencher, V.G. Dovi, Can. J. Biochem. 1 (1979) 313–318.
[67] S.W. Englander, N.R. Kallenbach, Q. Rev. Biophys. 16 (1984) 521–655.
[68] P.S. Kim, Methods Enzymol. 131 (1986) 136–156.
[69] R. Lumry, in: R.B. Gregory (Ed.), Protein–Solvent Interactions, Marcel Dekker, New York, 1994.
[70] K.S. Kim, J.A. Fuchs, C.K. Woodward, Biochemistry 32 (1993) 9600–9608.
[71] H.H. de Jongh, E. Goormaghtigh, J.-M. Ruysschaert, Biochemistry 36 (1997) 13603–13610.
[72] H.H. de Jongh, E. Goormaghtigh, J.-M. Ruysschaert, Biochemistry 36 (1997) 13593–13602.
[73] C. Vigano, A. Margolles, H.W. van Veen, W.N. Konings, J.-M. Ruysschaert, J. Biol. Chem. 275 (2000) 10962–10967.
[74] C. Gray, L.K. Tamm, Protein Sci. 6 (1997) 1993–2006.
[75] V. Cabiaux, J.T. Buckley, R. Wattiez, J.-M. Ruysschaert, M.W. Parker, F.G. van der Goot, Biochemistry 36 (1997) 15224–15232.
[76] X.M. Wang, R. Wattiez, M. Mock, P. Falmagne, J.-M. Ruysschaert, V. Cabiaux, Biochemistry 36 (1997) 14906–14913.
[77] E. Goormaghtigh, H.H. de Jongh, J.-M. Ruysschaert, Appl. Spectrosc. 50 (1996) 1519–1527.
[78] A. Menikh, M.T. Saleh, J. Gariepy, J.M. Boggs, Biochemistry 36 (1997) 15865–15872.
[79] L. Vigneron, J.-M. Ruysschaert, E. Goormaghtigh, J. Biol. Chem. 270 (1995) 17685–17696.
[80] N. Sonveaux, A.B. Shapiro, E. Goormaghtigh, V. Ling, J.-M. Ruysschaert, J. Biol. Chem. 271 (1996) 24617–24624.
[81] E.I. Pécheur, I. Martin, A. Bienvenue, J.-M. Ruysschaert, D. Hoekstra, J. Biol. Chem. 275 (2000) 3936–3942.
[82] V. Grimard, C. Vigano, A. Margolles, R. Wattiez, H.W. van Veen, W.N. Konings, J.-M. Ruysschaert, E. Goormaghtigh, Biochemistry 40 (39) (2001) 11876–11886.
[83] C. Vigano, V. Grimard, A. Margolles, E. Goormaghtigh, H.W. van Veen, W.N. Konings, J.-M. Ruysschaert, FEBS Lett. 530 (2002) 197–203.
[84] J.M. McCune, L.B. Rabin, M.B. Feinberg, M. Lieberman, J.C. Kosek, G.R. Reyes, L. Weissman, Cell 53 (1988) 55–67.
[85] J.P. Moore, B.A. Jameson, R.A. Weiss, Q. Sattentau, in: J. Bentz (Ed.), Viral Fusion Mechanisms, CRC Press, Boca Raton, FL, 1993, pp. 21–54.
[86] C.C. Broder, D.S. Dimitrov, Pathobiology 64 (1996) 171–179.
[87] P.R. Clapham, A. McKnight, R.A. Weiss, J. Virol. 66 (1992) 3531–3537.
[88] R.L. Damico, J. Crane, P. Bates, Proc. Natl. Acad. Sci. U. S. A. 95 (1998) 2580–2585.
[89] E.A. Berger, P.M. Murphy, J.M. Farber, Annu. Rev. Immunol. 17 (1999) 657–700.