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SARS E protein in phospholipid bilayers: an anomalous X-ray reflectivity study

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Abstract

We report on an anomalous X-ray reflectivity study to locate a labelled residue of a membrane protein with respect to the lipid bilayer. From such experiments, important constraints on the protein or peptide conformation can be derived. Specifically, our aim is to localize an iodine-labelled phenylalanine in the SARS E protein, incorporated in DMPC phospholipid bilayers, which are deposited in the form of thick multilamellar stacks on silicon surfaces. Here, we discuss the experimental aspects and the difficulties associated with the Fourier synthesis analysis that gives the electron density profile of the membranes.

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There is an increasing need in biomedical research for techniques capable of probing the structure and conformation of proteins and peptides in fluid lipid membranes such as viral ion channels and pharmaceutically relevant membrane proteins. The use of modern X-ray and neutron reflectivity techniques as tools to study biomimetic membranes is currently explored. These systems are composed of highly oriented lipid bilayers containing peptides or proteins, at controlled peptide-to-lipid ratios (P/L), which self-assemble on silicon surfaces, and can be swollen in water or water vapor [1]. We report the use of anomalous reflectivity [2] to gain structural sensitivity to a specific iodine-labelled phenylalanine residue in E protein of severe acute respiratory syndrome (SARS) coronavirus. Just as in crystallography, the labelling of individual residues in combination with anomalous X-ray scattering can provide important structural constraints, e.g. to locate an

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individual amino acid with respect to the lipid bilayer. Here, we have used the well-known phospholipid model system dimyristoyl-sn-glycero-3-phospatidycholine (DMPC). The transmembrane part of the SARS E protein was synthesized by the Fmoc method [3], corresponding to the residues 7–38 of the protein [4]. Two different synthetic peptides were made: an unlabelled peptide and one containing iodine at position 23 (i.e., Phenylalanine) of the sequence. The lipid and peptide fractions were co-dissolved in organic solvent and deposited on pre-cut and cleaned silicon wafers by spreading the organic solution [5]. The procedure results in a very low membrane mosaicity, which is a prerequisite to apply X-ray reflectivity.

While we have previously determined the iodine position from the comparison of electron density profile $\rho(z)$ of samples with unlabelled peptides to those of labelled peptides [3,6], we investigate here whether one can obtain the same results on a single sample, by using several photon energies around an absorption edge of the iodine label. Note that it is also possible to draw conclusions from the comparison of two multilamellar samples with and without the label, but small differences in the two samples such as number N of bilayers and/or different defect densities may influence the achievable accuracy. Here, we explore whether anomalous reflectivity is a reasonable alternative. The anomalous dispersion effect near the absorption edge of an atom is accounted for by the complex atomic scattering factor expressed as $f(q,E) = f0(q) + f1(E) + \mathrm{i}f2(E)$, where q and E are the wavevector and the incident energy, respectively; $f0(q)$ corresponds to the scattering factor of the atom at energies sufficiently far from the absorption edge, and $f1(E)$ and $f2(E)$ are the real and the imaginary parts of the anomalous dispersion terms. These last two terms lead to a sensitivity of the reflectivity to the excited (resonant) atomic label. Differences in the reflectivity curves obtained at a set of energies $R(q_z,E)$ then can be analyzed in terms of corresponding differences in the electron density profiles $\rho(z,E)$ [7]. For example, measuring close to and far from the iodine L_{III} edge at $E_{\mathrm{L}} = 4.5545\,\mathrm{keV}$, yields a difference electron density profile for the iodine atom as $\rho_{\mathrm{I}}(z,E) = \rho_E(z) - \rho_{E_{\mathrm{L}}}(z)$.

Experiments were carried out at the ID1 beamline of the European synchrotron radiation facility (ESRF) in Grenoble, France. While scanning the photon energy around E_{L} in steps of 0.5 eV by a double Si(1 1 1) monochromator, the fluorescence spectrum of the sample was measured at fixed angle of illumination α_i, using a silicon drift detector (X-flash, Roentec) with 150 eV energy resolution, which is positioned vertically above the sample. A fluorescence curve $I_{\mathrm{f}}(E)$ directly proportional to $f2(E)$ was obtained by defining the region of interest in the spectrum corresponding to the $I\ L_{\mathrm{III}}$ fluorescence. This procedure using the sample, proved to be more practical than the absorption measurement of NaI solutions, which can also be used to determine the absorption coefficient $\mu(E)$, from which $f2(E)$ is obtained by the optical theorem $f2(E) = -(k\mu/4\pi\rho r_0)$ [8]. In the present case, we scaled the measured curve of $f2(E)$ to the tabulated values [9], as shown in Fig. 1(a) and combined it with the tabulated values to obtain a data set of higher precision at the absorption edge. The spectrum away from the absorption edge was normalized (absolute scale of electrons equivalents) to the standard tabulated values. The $f2$ values were then extrapolated over the entire energy range. The real part $f1$ which dominates the observed dispersion is then calculated from the usual Kramers–Kroning (KK) relation according to

$$f1(E) = \frac{2}{\pi} P \int \frac{E'^2 f2(E)}{E'^2 - E^2}\,\mathrm{d}E', \tag{1}$$

where P in the front of the integral stands for 'principal value', which was carried out by numerical calculations (using a modified verion of a program kindly provided by T. Schülli). The resulting curve obtained from the KK integral is shown in Fig. 1(b) and exhibits a significant contrast of $\simeq (42-34)/42 = 0.19$ for the $I\ L_{\mathrm{III}}$ edge. For the contrast variation measurement, the following five photon energies were chosen: $E1 = 4.3975\,\mathrm{keV}$, $E2 = 4.5175\,\mathrm{keV}$, $E3 = 4.5435\,\mathrm{keV}$, $E4 = 4.5545\,\mathrm{keV}$, and $E5 = 4.5675\,\mathrm{keV}$. It is apparent from Fig. 1 that the energies marked by $E1$ and $E4$ are optimal for the anomalous contrast, because the change in $f1$ between these two points