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Detergent organisation in solutions and in crystals of membrane proteins

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Abstract

The use of neutron scattering in studying the organisation of detergents in pure micelles, in protein/detergent mixed micelles and in crystals of membrane proteins, is reviewed. Small angle scattering has been used to study the size, shape and composition of pure and mixed protein/detergent micelles as well as the effects of adding small amphiphiles. The technique of contrast variation applied to single crystals is described and its application to the determination of the organisation of detergent in single crystals of membrane proteins is discussed. A better understanding of protein/detergent interactions should help in producing crystals of membrane proteins more easily as well as giving clues to the nature of protein/lipid interactions in vivo.

Keywords: Membrane proteins; Detergents; Amphiphiles, small; Neutron crystallography; Small angle scattering

1. Introduction

There has been a rapid increase in structural studies of membrane proteins since the successful crystallisation of the photosynthetic reaction centre from *Rhodopseudomonas Viridis* [1] and matrix porin, also known as ompF [2]. The X-ray crystal structures were determined for two related bacterial reaction centres in the 1980s [3,4] and more recently for three related porins [5–7]. Despite these dramatic advances the number of structures solved is still not very great and has been limited by the few proteins to have been successfully crystallised. This is perhaps not surprising as membrane proteins possess a large hydrophobic surface area, which is probably not well adapted to the formation of precise intermolecular contacts and has to be at least partially solubilised by a detergent-belt which must be accommodated in the crystal lattice. Detergents have long been used for the extraction of membrane proteins from their normal lipid membrane environment, and it was recognised that only rather mild non-ionic detergents could be used without denaturing the protein.

In order to understand better the crystallisation of membrane proteins a first step is to understand the behaviour of detergent solutions in the absence of membrane proteins. Here we will review the use of neutron scattering methods to determine the structures of the micellar phase from which membrane proteins are usually crystallised.

Another approach to understanding the role of detergents in membrane protein crystallisation is to study the final product – the membrane protein crystal. However, in the few X-ray structures which have been

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solved to date, little or no detergent has been located. This is due to the fluidity and low electron density of the detergent phase producing insufficient contrast with respect to the aqueous phase. Again neutron diffraction from membrane protein crystals has been able to show, through the use of D_2O/H_2O contrast variation, the organisation of the detergent phase. Recent results in this field will be discussed.

Rather than provide a comprehensive review of this field, the aim of this article is to illustrate, through particular examples, the role of neutron scattering in understanding membrane protein/detergent interactions and, particularly, their role in membrane protein crystallisation.

2. Contrast variation in protein/detergent systems

The scattered intensities from a solution or from a crystal are related to the contrast: i.e. the difference in scattering density between the molecule and the solvent. Table 1 gives an overview of the scattering densities of detergents commonly used in membrane protein studies. The volumes of the alkyl chains can be calculated as explained in the caption of Table 1; volumes of the head groups can be reasonably estimated from the chemical formula. The total volume of a detergent molecule can be deduced from solution scattering experiments or simply from the partial specific volume determined by densimetry. The hydrophobic tails contain a larger amount of hydrogen than the head groups, having therefore a lower match point. This difference in match point is in some cases quite important, for example, for dodecyl maltoside where it varies from 48% D_2O for the head groups to 2% D_2O for the tails. In studies of protein/detergent complexes, it is of interest to differentiate between tails and head groups in order to identify which part of the detergent interacts with protein. This can be achieved if the two detergent domains are sufficiently distinct compared to the observed resolution and if the contrast is strong enough. The contrast between head and tail can be enhanced by specific deuteration as shown in Table 1. If the resolution is too low to distinguish the domains separately, one will deal with the mean match point of the whole detergent molecule. Fig. 1 represents the scattering densities of solvent, a typical protein and two different detergents. It shows how the contrast of protein, whole detergent, detergent tail or detergent head can be changed by varying the D_2O content of the solvent.

3. Detergent organisation

3.1. Pure detergent micelles

Detergents used in the solubilisation of membrane proteins are almost invariably non-ionic or zwitterionic, although bile salts, which carry a net negative charge, were in the past widely used and are still commonly used for the purification of certain membrane proteins. All detergents which are suitable for solubilisation are not necessarily effective for crystallisation as will be seen later.

The solution behaviour of non-ionic detergents has been considered by several authors and, in particular, recently by Zulauf [8]. These properties are largely determined by the amphiphilic nature of the detergent (surfactant) molecules, having a hydrophilic head and a hydrophobic tail. Fig. 2 is a schematic phase diagram for a non-ionic detergent showing the different phases existing as a function of temperature and solution concentration. Note that addition of nondetergent components to a pure detergent solution can change the phase diagram substantially. For example the addition of PEG (a commonly used precipitant in crystallisation) may shift the phase separation boundary to lower temperatures, or the addition of small alcohols may raise the same boundary. The schematic diagram shown in Fig. 2 is rather simple. Such diagrams may be much more complicated and may change radically with a very small change in the hydrophobic/hydrophilic balance. Early reports of membrane protein crystallisation indicated that crystals were usually observed under conditions corresponding to the phase separation area of the phase diagram with a tendency for the crystals to occur in the detergent-rich phase. Subsequently, however, crystals have been observed more frequently to grow from solutions of a single micellar phase. The concentration of detergent within a protein crystal may, however, be much higher than that of the solution from which it has crystallised. Garavito [9] calculated that ompF crystals contain 30–40% (w/v) of octyl glucoside depending on the crystal form and if one considers only the nonprotein volume then this figure becomes 40–60%. This could place the crystallising conditions

Table 1
Scattering densities for some detergents used in membrane protein studies

Detergent	Volume [a] (Å^3)	Scattering length density (10^{-14} cm A^{-3})	Match point % D_2O
DDAO			
(N-decyl-N,N-dimethylamine-N-oxide)			
protonated head	81.0	0.748	18.8
protonated C_{10} tail	296.4	−0.406	2.6
whole molecule protonated	377.4	−0.158	5.8
deuterated C_{10} tail	296.4	6.972	108.1
whole molecule (protonated head group, deuterated tail)	377.4	5.636	88.9
LDAO			
(N-dodecyl-N,N-dimethylamine-N-oxide)			
protonated head	81.0	0.748	18.8
protonated C_{12} tail	350.2	−0.392	2.4
whole molecule protonated	431.2	−0.179	5.5
deuterated C_{12} tail	350.2	7.041	109.1
whole molecule (protonated head group; deuterated tail)	431.2	5.859	92.1
C12MS			
(dodecyl-β-D-maltoside)			
protonated head	358.4	$1.820 + 2.03x$ [b]	48.2
protonated tail	350.2	−0.391	2.4
whole molecule	708.6	$0.725 + 1.028x$	21.7
ES12H			
(1-dodecanoylpropanediol-3-phosphoryl-choline)			
protonated head	263.3	0.856	20.3
protonated tail	447.4	0.024	5.4
whole molecule protonated	710.7	0.355	13.2
Triton X100			
protonated head	633.4	$0.687 + 0.164x$	17.5
protonated tail	378.6	0.385	13.5
whole molecule	1012.0	$0.573 + 0.103x$	16.5
C_8E_4			
(octyl tetraoxyethylene)			
protonated head	267.4	$0.696 + 0.389x$	19.1
protonated C_8 tail	236.7	−0.440	1.8
whole molecule protonated	504.1	$0.163 + 0.207x$	9.3
deuterated C_8 tail	236.7	7.037	109.0
whole molecule (protonated head group, deuterated tail)	504.1	$3.670 + 0.389x$	64.4
β-OG			
(octyl-β-D-glucopyranoside)			
protonated head	180.3	$1.859 + 2.310x$	52.0
protonated C_8 tail	236.7	−0.440	1.8
whole molecule protonated	417.0	$0.554 + 0.999x$	18.6

[a] Molecular volumes are calculated from experimentally determined partial specific volumes. For head groups and tails, volumes are reasonable estimates based on their chemical nature except in the case of alkyl chains where they are calculated according to Tanford's formula [34]: $v = 27.4 + 26.9\ n_C$, where n_C is the number of carbon atoms.
[b] x, mole fraction D in solvent.

in one of the mesophase (cubic, hexagonal or lamellar) regions of the phase diagrams.

A first step in understanding detergent organisation is to investigate micellar structures. Detergent micelles have been investigated by a variety of methods including dynamic light scattering, X-ray small angle scattering and neutron small angle scattering. Through small angle neutron scattering (for a review see [10]) it is possible to measure the shape and molecular mass of the micelles and to measure the variation of these

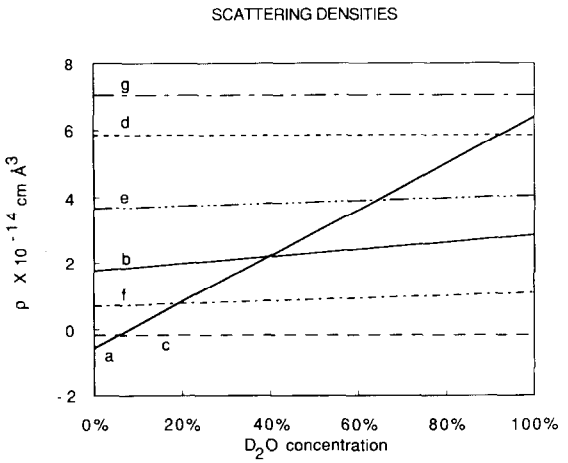

Fig. 1. Scattering densities as a function of the D_2O concentration in the solvent, are represented for: a, water; b, a typical protein; c, completely protonated LDAO; d, LDAO with a protonated head group and a deuterated tail; e, C_8E_4 with a protonated head goup and a deuterated tail; f, the protonated head group from C_8E_4; g, the deuterated tail from C_8E_4. The values are taken from Table 1. The detergent is matched when it has the same scattering density as the solvent. Lines f and g show that with specific deuteration, one can separately visualise the head group and the tail.

parameters as a function of the local environment (temperature, salt concentration, etc.). The methodology of such a determination is well described in the review by Chen [11].

The molecular mass of the micelle, which gives the aggregation number, may be estimated from the normalized neutron scattering intensity at zero angle using the expression of Jacrot and Zaccai [12].

$$\frac{I(0)}{I_{inc}(0)} = f\frac{4\pi T_s}{1-T_w} CN_A t \times 10^{-3} \left(\frac{1}{M_r}(\Sigma b - \rho_s V)\right)^2 M_r,$$

where $I(0)$ is the scattered neutron intensity at zero angle, $I_{inc}(0)$ the scattering (essentially incoherent) from a sample of water under the same geometrical conditions, f is a wavelength dependent correction factor, T_s the sample transmission, T_w the transmission of water, C the sample concentration in mg/ml, N_A Avogadro's number and t the sample cell thickness. $\Sigma b/M_r$ is the scattering length density of the micelle per unit molecular weight which is the same as that for an individual molecule and may thus be computed from the scattering length of the individual atoms and the detergent partial specific volume. r_s is the scattering length density of the solvent and V/M_r is computed from the partial specific volume, leaving M_r the micelle mass, the only unknown.

Many detergents, in particular their hydrophobic tails, have a scattering density close to that of pure H_2O. It is advisable in these cases to make the molecular weight estimate from a measurement in D_2O. It is important in this case to be aware of the importance of knowing precisely the partial specific volume of the detergent. A 1% error in this parameter can lead to a 2% error in the micelle mass.

The geometry of micelles can be deduced using small angle scattering and molecular geometry constraints such as maximum chain length. Chen [11] has reviewed the determination of micelle shapes for both charged and uncharged detergents usually from data in concentrated solutions. Here it is necessary to separate the total scattered signal into the interference and the form factor. The interparticle structure factor can have a significant influence on the total scattering in concentrated solutions ($\geqslant 10$ mg/ml) or for charged micelles. For non-ionic micelles it is possible to determine the form factor directly by scattering from dilute solutions ($\leqslant 10$ mg/ml). In ideal cases the shape may be deter-

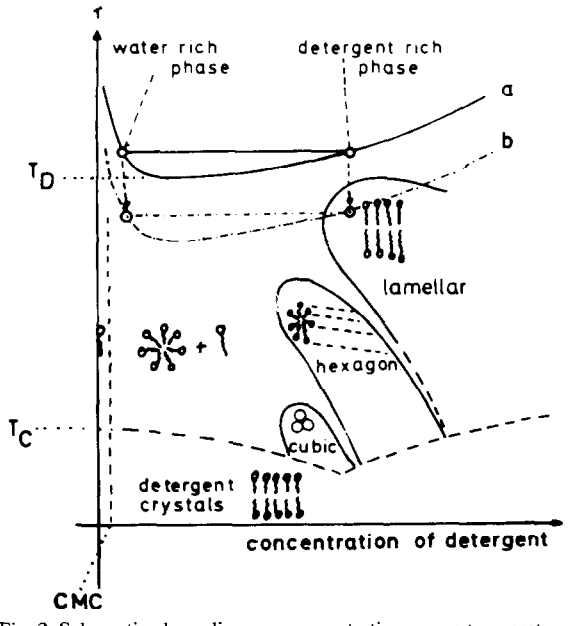

Fig. 2. Schematic phase diagram, concentration versus temperature, for a non-ionic detergent/water system. The phase separation boundary is labelled a and may shift to b on addition of, for example, PEG. The liquid crystal phases, cubic, hexagonal and lamellar, are usually found at detergent concentrations above $\approx 30\%$.

mined by model fitting to the extended scattering curves assuming spherical or simple ellipsoidal shapes. In many cases the headgroup and hydrophobic tail may have significantly different scattering length densities, in which case H_2O/D_2O contrast variation can be used to determine the radial locations of headgroup and tail. If the head group and tail have similar scattering length densities it may be possible to specifically deuterate one or the other as was the case for LDAO [13]. For some detergents producing rather small micelles it is not possible to measure the data from dilute solutions to high enough Q-values to fit models. In these cases it may only be possible to measure data in the Guinier range and hence derive radii of gyration. This may, however, still be sufficient to establish the difference between spherical and ellipsoidal micelles if one uses also reasonable geometrical constraints, such as the fact that the smallest radius of the particle cannot be greater than the fully extended molecular length. This is the case for micelles of dodecyl maltoside [14]. The dimensions and aggregation numbers of a number of non-ionic or zwitterionic micelles determined by neutron scattering are listed in Table 2. It is important to be aware of the fact that micelle structure can be extremely sensitive to temperature and that the parameters quoted are only correct at the specified temperature.

The size and shape of protein/detergent mixed micelles are of critical importance when attempting to crystallise membrane proteins. Almost no systematic studies of such micelles under crystallising conditions have been carried out, although a very recent study by Gast et al. [15] has used turbity measurements to study the binding of LDAO to the photoreaction centre from

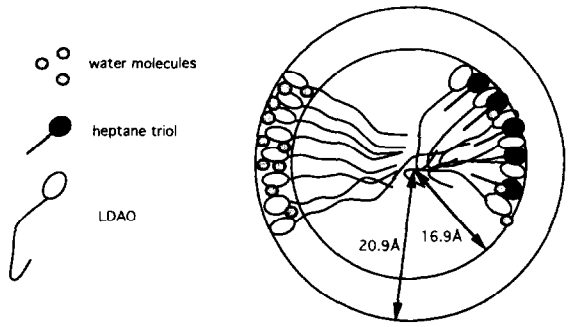

Fig. 3. The effect of heptane-1,2,3-triol on the size and shape of LDAO micelles. On addition of 10% heptane-1,2,3-triol to 1% LDAO the micelle radius decreases. The number of LDAO molecules per micelle decreases from 69 to ca. 34, whilst some 23 heptane-1,2,3-triol molecules are incorporated and almost all the water hydrating the head groups is excluded. The figure is only schematic and is not to scale. Data from [13].

Rhodopseudomonas viridis. An important observation made by Michel [16] was that photosynthetic reaction centres from *Rhodopseudomonas viridis*, as well as some other proteins, would only crystallise in the presence of small amphiphilic molecules as well as the solubilising detergent. The effect of one of these small amphiphiles, heptane-1,2,3-triol (HP) on the size and shape of LDAO micelles has been investigated using small angle neutron scattering [13]. In this case LDAO was available both in its hydrogenated form and with its hydrophobic tail deuterated. When HP is added to hydrogenated LDAO micelles in D_2O then any HP incorporated in the micelles contributes to the scattering and the total micelle mass can be calculated. However, if HP is added to deuterated micelles in H_2O the HP is virtually invisible and the scattering is due only to the contrast between the LDAO and H_2O. In this

Table 2
Some structural parameters for micelles of non-ionic or zwitterionic detergents established by neutron scattering

Detergent [a]	Chemical formula	Temperature (°C)	Aggregation No.	Ellipsoid half axes or spherical radius (Å)	Ref.
LDAO	CH_3-$(CH_2)_{11}$-$N^+(CH_3)_2$-O^-	20	69	20.7	[14]
C12MS	CH_3-$(CH_2)_{11}$-$(C_6H_{10}O_6)_2H$	16	130	26.8×28.8×28.8	[14]
Triton X100	$(CH_3)_3CH_2C(CH_2)$-(C_6H_4)-O-$[CH_2$-$CH_2]_n$-H [b]	16	89	45×35×35 [c]	[18]
C8E5	CH_3-$(CH_2)_7$-$[O$-CH_2-$CH_2]_5OH$	7	68	23	[35]
ES12H	$CH_3(CH_2)_{10}$-$C(=O)O(CH_2)_3$-O-$P(=O)_2$-O-(CH_2)-$N^+(CH_3)_3$	16	68	23.9	[14]

[a] For full name, see Table 1.
[b] On average, $n = 10$.
[c] Consistent with the radius of gyration given in Ref. [18] and length of fully extended molecule.

way it was possible to monitor the size, shape and composition of the micelles as a function of the HP content of the solvent. The results are summarised in Fig. 3. The effect of the heptane triol is to partially insert into the LDAO micelle at the same time increasing the radius of curvature and diminishing the size of the micelle. The studies by Gast et al. [15] have shown that the number of molecules of LDAO bound to reaction centres from *Rhodopseudomonas viridis* is reduced by a factor of two in the presence of 5% HP. These observations support Michel's hypothesis [16] that the effect of small amphiphiles in crystallisation is to reduce the size of the detergent micelles favouring protein–protein interactions which are essential for the formation of ordered crystals.

3.2. *Detergent/protein complexes in solution*

Binding of detergents to membrane proteins has been studied extensively using chromatographic procedures with radioactively labelled detergents. Some of the advantages and difficulties of this method are discussed in ref. [17]. Contrast variation studies of protein/detergent complexes in solution by small angle neutron scattering can yield information which is otherwise unobtainable or difficult to obtain using other techniques. The measurement of protein molecular weight is carried out very simply in a way rather similar to that described for pure micelles [12]. The most precise way in which to perform this measurement is to carry out separate experiments on pure detergents and protein/detergent complexes as a function of contrast. In this way the buffer composition at which the detergent forward scattering is zero can be determined experimentally. It is then possible by interpolation to extract the intensity at zero angle for the complex in the solvent where the detergent is matched out. This is well illustrated in the study of active Photosystem I (PSI) preparations solubilised in Triton X-100 [18]. In order to maintain the integrity of the protein the buffer contained 20% glycerol which would introduce considerable uncertainty in any calculation of the detergent match point. The experimental determination allowed the forward scattering of the protein in the complex to be determined at the correct contrast. This demonstrated that active preparations of PSI were monomeric in Triton X-100.

Another important use of contrast variation is to determine the extent of detergent binding in protein/detergent complexes. This is calculated from the match point of the complex using the expression:

$$x = \frac{(\rho_P - \rho_s)\bar{v}_P}{(\rho_P - \rho_s)\bar{v}_P - (\rho_D - \rho_s)\bar{v}_D},$$

where x is the mass fraction of detergent in the complex, $\bar{v}_P$ the partial specific volume of the protein, $\bar{v}_D$ the partial specific volume of the detergent, ρ_P the scattering length density of the protein at the match point of the complex, ρ_D the scattering length density of the detergent at the match point of the complex, and ρ_s the scattering length density of solvent.

This kind of measurement is illustrated also in the PSI study [18] and also in a study of porin from *E. coli* solubilized in C_8E_4 [19]. In this case, fully deuterated protein was used to increase the contrast between protein and detergent. An important result was that the amount of detergent associated with the protein varied with the temperature: 180 molecules of C_8E_4 per porin trimer at 6°C and 310 per trimer at 29°C. This was interpreted as there being a complete monolayer covering of the protein hydrophobic surface at 6°C whilst the increased number of detergent molecules associated at 29°C was postulated to be due to the attraction of free micelles to the protein/detergent complex, rather than a modification of the initially bound monolayer.

Small angle scattering using contrast variation can also yield information on the shapes of protein/detergent complexes. Here, however, it should be remembered that the concept of a 'contrast match point' is valid only for scattering at zero angle. At non-zero angles a component whose zero angle scattering is matched may still contribute significantly. This is a more serious limitation for detergents than for proteins and nucleic acids as the heads and tails of these molecules may have very different scattering length densities (see Table 1) and they are usually spatially well separated. An example where such a study was successful was in the case of rhodopsin [20] which can be solubilised in LDAO micelles which are in fact rather homogeneous.

In future studies we can envisage obtaining more reliable shape information of protein/detergent complexes by modelling using the known shape of the membrane protein from X-ray crystallographic studies.

3.3. Crystalline protein/detergent complexes

The study of the detergent phase in single crystals of membrane proteins requires the use of low resolution neutron crystallography, a technique which is not widely known. For this reason we describe here the basic principles of the method.

Principles of low resolution crystallography

Crystals of biological macromolecules or assemblies of such molecules sometimes contain disordered regions. The disorder can be related to the macromolecule itself, for instance nucleic acids in viruses, or the crystalline assembly can contain less ordered regions. In both cases, high resolution X-ray diffraction experiments lead to structural information on the highly ordered parts only. For a better understanding of the relationship between biological function and structure, the less ordered domains may play an important role due to their flexibility and dynamic properties. Low resolution information is obtained from diffraction at very small Bragg angles. These reflections are usually difficult to record, especially when working with strong X-ray sources where it is important to protect the detector against the main beam. To overcome this technical problem one may increase the wavelength and therefore increase the Bragg angle. Long wavelength X-rays induce strong absorption and thus damage the crystals rapidly. On the contrary, neutrons do not present these absorption effects and hence do not induce radiation damage even during exposures of several weeks. Neutron diffraction using long wavelengths is a powerful technique for obtaining low resolution structural information and is complementary to the high resolution information gained from X-ray diffraction. In addition to the advantages of long wavelengths one can take advantage of the difference in scattering lengths between protons and deuterons to perform contrast variation experiments in the crystals. The fact that H_2O and D_2O are chemically almost identical, makes it possible to exchange the solvent in the crystals for a deuterated solvent without affecting the crystalline arrangement.

Low resolution neutron diffraction experiments are carried out at ILL, Grenoble on a specially designed diffractometer: DB21. DB21 was built jointly by ILL and the European Molecular Biology Laboratory (EMBL) and is installed on a cold neutron source. A 7.5 Å wavelength beam is selected by a potassium intercalated pyrolytic graphite monochromator. Crystals are mounted on a standard 4-circle device. Diffraction intensities are measured on a two-dimensional detector [21]. The diffractometer operates under an Ar atmosphere to minimise the background scattering from air. The experimental set-up allows measurement of diffraction data from crystals with unit cells up to 1000 Å to a resolution from infinity to $\simeq 12$ Å. This set-up is presently being improved with an automatic monochromator changer affording an easy wavelength selection. The accessible resolution would then be increased by selecting a 4.5 Å wavelength obtained from a standard pyrolytic graphite monochromator.

As neutron Bragg reflections from these crystals are relatively weak, it is therefore of primordial importance to work on a powerful neutron cold source. Crystal sizes are typically around 0.5 mm in each dimension. Data collection for one data set to 12 Å resolution lasts about one week. However, it has been possible to collect usable diffraction data from smaller crystals such as ribosomal particle crystals. These crystals are thin plates of $0.5 \times 0.5 \times 0.05$ mm, and data sets to 30 Å resolution could be collected within 3–5 weeks [22]. These long data collection periods are possible because of the absence of radiation damage to the crystals in the neutron experiments and the stability of the instrumental set-up. For contrast variation experiments it is important to collect a very high fraction of the data, particularly at the lowest resolution.

Data treatment presents some difficulties due to the low resolution of the data, in particular the small number of strong spots makes the use of automatic orientation programs difficult or even impossible. A first orientation matrix is calculated by an interactive graphics program working on a Evans and Sutherland PS station (KEA; P. Metcalf, unpublished) which displays the spots as well as the crystallographic lattice in reciprocal space. The lattice is then rotated until it superimposes with the spots. This program is a direct way visualising the reflections in reciprocal space independently of any lattice information and is therefore very helpful in checking the quality of the crystals.

The determination of the structure requires, as for X-rays, a solution to the phase problem. However, contrast variation places strong constraints on the possible phases.

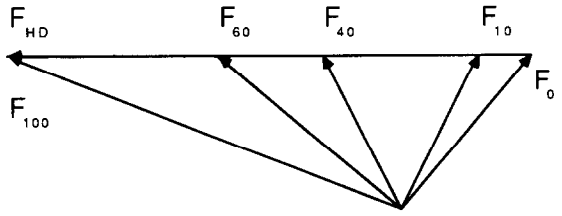

Fig. 4. Argand diagram representing the variation of the complex structure factors for one reflection with the D_2O concentration. This D_2O diagram gives the relative variation, and thus the relative phases of each concentration. The absolute phases are determined, for example, by knowing part of the structure from high resolution X-ray data.

The scattering density $\rho(r)$ is a linear function of the D_2O concentration of the crystal mother liquor x (Fig. 1):

$$\rho_x(\boldsymbol{r}) = \rho_o(\boldsymbol{r}) + x\Delta\rho(\boldsymbol{r}) \quad (1)$$

The Fourier transformation of expression (1) gives the relation of the complex structure factor versus x:

$$\boldsymbol{F}_x(h,k,\ell) = \boldsymbol{F}_o(h,k,\ell) + x\boldsymbol{F}_{HD}(h,k,\ell) \quad (2)$$

The diffracted intensity is thus a quadratic function of x:

$$I_x(h,k,\ell) = |F_o(h,k,\ell)|^2 + 2x\cos\phi|F_o||F_{HD}| + x^2|F_{HD}|^2 \quad (3)$$

In the special case of centric reflections, relation (2) is simplified to:

$$F_x(h,k,\ell) = F_o(h,k,\ell) + xF_{HD}(h,k,\ell). \quad (4)$$

Eq. (2) can be represented by a vector diagram (Fig. 4). The determination of the parabolic constants in Eq. (3) requires the collection of at least four different contrast diffraction data. The solvent exchange to deuterated or partially deuterated solvent results from soaking of the crystals for 2–3 weeks. This soaking time takes into account not only the exchange of the solvent but also the exchange of accessible hydrogens involved in O–H or N–H bonds [23]. The centric reflections are first used to scale the experimental data sets obtained for different D_2O concentrations together. Then, one can determine the parabolic constants for each general reflection [24].

How can the relative variation of the complex structure factors be used? Fig. 4 shows clearly that the determination of the parabola parameters allows the relative locations of the $\boldsymbol{F}(h,k,\ell)$, which means that knowing the $\boldsymbol{F}(h,k,\ell)$ for one D_2O concentration one can calculate the structure factors at any contrast. A typical application of this method is the determination of a disordered part in a crystal where the highly ordered part is already known to atomic resolution from X-ray diffraction experiments. For example, in the case of a membrane protein, a usual protonated detergent will be matched out at 10–20% D_2O solvent concentration (Table 1). This means that the neutron structure factors at this contrast can be calculated directly from the coordinates of the protein atoms. Performing a contrast variation experiment as described previously will give access to structure factors at any contrast. In particular, a 40% D_2O contrast will match out the protein (Fig. 1) and a density map obtained from these structure factors will represent the remaining part of the crystal component, i.e. the detergent. The calculation of the optimal density maps at any contrast from the experimental diffraction data and the X-ray model is developed in ref. [33]. Similar experiments have been performed with a lipoprotein, lipovitellin [25], and with viruses [26].

Recently a new approach has been developed. In the case where no X-ray information is available, attempts are being made to obtain phase information by combining direct methods with form factor or envelope information from EM or solution scattering data [27,28]. It is also interesting to note that a first modelling of the *E. coli* matrix porin from neutron diffraction data was done without high resolution information [29]. This modelling was based on electron microscopy results describing the porin as a trimer of cylinders. Using low resolution neutron diffraction data these trimeric cylinders were located in the crystal at positions which were later confirmed by high resolution X-ray diffraction studies.

Detergent organisation in membrane protein crystals

At the present time four membrane protein crystals have been studied by low resolution neutron crystallography. The first detergent structures were observed in bacterial photosynthetic reaction centres from *Rhodopseudomonas viridis* [30] and *Rhodopseudomonas sphaeroides* [31]. More recently the detergent domains were localised in *E. coli* matrix porin crystals (in preparation) and in *Rhodobacter capsulatus* porin (in preparation). From these data, some observations can be made concerning the detergent/protein interactions and the detergent phase itself. In the photoreac-

tion centre, the detergent appears as a continuous three-dimensional structure. Detergent rings surround the protein and are connected by short cylindrical detergent bridges. The thickness of the detergent rings in the direction of the transmembrane α-helices is ≈30 Å. In the case of the reaction centre from *Rhodobacter sphaeroides* [31], the authors interpret this as suggesting that the detergent is organised around the protein molecule like an ellipsoidal micelle rather than a bilayer. A direct determination of the location of heads and tails could be made by carrying out such experiments with partially labelled detergent as shown in Table 1. In the *Rhodobacter capsulatus* porin the detergent forms a planar structure containing the porin trimers. Within the plane there are no connections between the protein trimers and from one plane to the next there are no connections between detergent phases. It appears very similar to a stacking of membranes connected by protein/protein interactions, except that as this stage it is not possible to distinguish between a planar bilayer structure or a juxtaposition of micelles within the plane. Again this question could be solved by a specific labelling of the head groups or the tails.

4. Conclusions

We have demonstrated how neutron scattering using contrast variation is a powerful technique for understanding detergent organisation both alone and complexed with membrane proteins. Both small angle scattering and single crystal diffraction can play important roles. The knowledge of further membrane protein low resolution crystal structures will lead to a better understanding of membrane protein crystallisation, in terms of the choice of the detergent, but it should also provide an analysis of the protein/detergent contacts for a better understanding of the protein/lipid interactions in in situ membrane proteins. For such goals, more precise contrast variation experiments, labelling separately the head group and the hydrophobic tail of the detergent are proposed. This type of experiment has already been performed in the case of the *E. coli* matrix porin (ompf) crystals for which the detergent tail was totally deuterated. It would also be of interest to study membrane protein crystals obtained in the presence of lipids instead of detergent [32] to better mimic a real biological membrane. Specific deuteration could also help in locating additive molecules such as heptane-1,2,3-triol, which play an important role in obtaining the crystals and which cannot be located as long as they have the same contrast as the detergent or the solvent as was the case in ref. [30].

In the future we can hope to extend the technique further, not only by more specific deuteration but also by extending the resolution of crystallographic data to ≈5–6 Å resolution. In solution studies it should be possible to offset the rather poor resolution by using crystallographic data from known protein structures to improve modelling of solution structures. And finally, if one can produce crystals with significant quantities of lipid bound to the membrane protein, neutron crystallography would be an excellent method for visualising protein/lipid interactions directly.

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