APPLICATIONS OF SYNCHROTRON RADIATION TO THE STUDY OF LARGE MOLECULES OF CHEMICAL AND BIOLOGICAL INTEREST

Proceedings of the Daresbury Laboratory Study Weekend,

27-28 January, 1979

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DARESBURY LABORATORY

1979

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CONTRIBUTED PAPERS:

FOREWORD

The purpose of the Study Weekend was to discuss the relevance of synchrotron radiation sources in the context of studies of "large molecules" of chemical and biological interest. The meeting was able to consider in detail several research projects in preparation for use with the SRS and to consider the merits of a number of quite new spectroscopic applications of synchrotron radiation. Perhaps most important was the opportunity for potential users of the SRS in fields in chemistry and biology to meet with existing users and other members of Daresbury Laboratory Staff in this truly multi-disciplinary research venture.

These proceedings are a record of the invited papers and contributed papers presented at the meeting, and are those submitted except for some standardisation and editorial changes and are printed in the order in which they arose at the meeting.

The success that the meeting achieved was undoubtedly due to the considerable efforts of the invited speakers and our thanks are due to them both for their talks and their co-operation in the preparation of these proceedings.

We thank the Laboratory and its Director, Professor A. Ashmore, for permission to hold the meeting and for the provision of financial support for the meeting itself and with the preparation of these proceedings.

We thank in particular Mrs. Shirley Lowndes and the Technical & Scientific Information Services staff, and Mrs. Christine Thompson from the Travel & Subsistence Section for their help with the planning and organisation of the Study Weekend. Dr. Kenneth Lea was, as ever, ready to lend a hand at all critical moments during the meeting and with the preparation of the proceedings and we are very grateful.

Finally we thank Miss Ann Haskayne for her excellent typing of the manuscripts and again Mrs. Shirley Lowndes and the TSIS staff particularly Mr. Geoff Berry and Mr. Ian Sharp for the preparation and production of this report.

I.H. Munro R.B. Cundall

OPTIMISATION OF ANOMALOUS SCATTERING AND STRUCTURAL STUDIES OF PROTEINS USING SYNCHROTRON RADIATION

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1. INTRODUCTION

The structure determination of a protein can utilise the method of multiple isomorphous replacement combined with anomalous dispersion of heavy atoms. The data collection from protein crystals faces the major problems of:

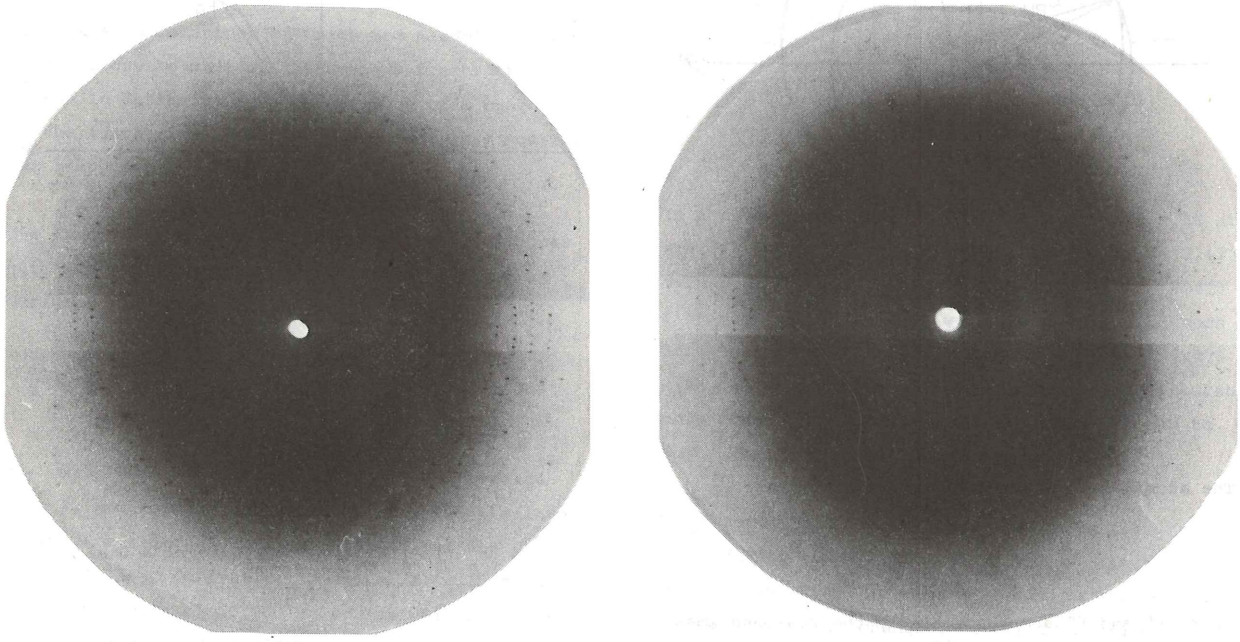
i) the inherent weakness of individual reflections,
ii) the voluminous amount of data to be collected,
iii) radiation damage.

The unique qualities of synchrotron radiation (SR) can make an impact on these problems as well as phase determination by multiple wavelength methods. In particular the outstanding properties of

i) high intensity in the hard x-ray region,
ii) tunability,
iii) small beam divergence

when compared to a conventional x-ray source.

Measurements from crystalline protein samples using SR can be conveniently divided into two classes. Firstly, small samples, large unit cells, the rapid collection of accurate high resolution data and dynamical studies can all benefit from the high intensity. Figure 1 compares native diffraction photographs from crystals of 6-phosphogluconate dehydrogenase, 6-PGDH (Adams et al[1]), taken on a Ni filtered GX6 and at the LURE Facility, Orsay, and shows a dramatic reduction of 26 in exposure time. A reduction in radiation damage was observed such that approximately twice as much data per crystal was obtained at the synchrotron illustrating that radiation damage was a function of exposure time rather than x-ray dose for these crystals.



(a) (b)

Fig.1 Native protein diffraction photographs taken on an Arndt-Wonacott oscillation camera from crystals of 6-phosphogluconate dehydrogenase C mount, $\Delta\phi = 1.5°$, circular beam of diameter 0.3 mm, resolution limit = 2 Å, crystal to film 60 mm, flatplate geometry. (a) GX6 Ni filtered radiation, 1.6 kW, exposure time = 10.4 hours IND G. (b) LURE 1.80 GeV 170 mA exposure time = 18.75 mins. Kodirex. Crystal used similar size in each case.

Secondly, an important extension of the classical methods of protein structure determination arises from use of the tunability of SR for optimisation of anomalous scattering and subsequent phase determination. This paper will concentrate on this area of application.

2. ANOMALOUS SCATTERING AND ITS USE IN CRYSTALLOGRAPHY

For acentric reflections the use of a single isomorphous derivative (SIR) leaves a phase ambiguity (fig.2) which can only be resolved by using anomalous scattering information or a second derivative. In the absence of errors either of these would be sufficient (fig.3).

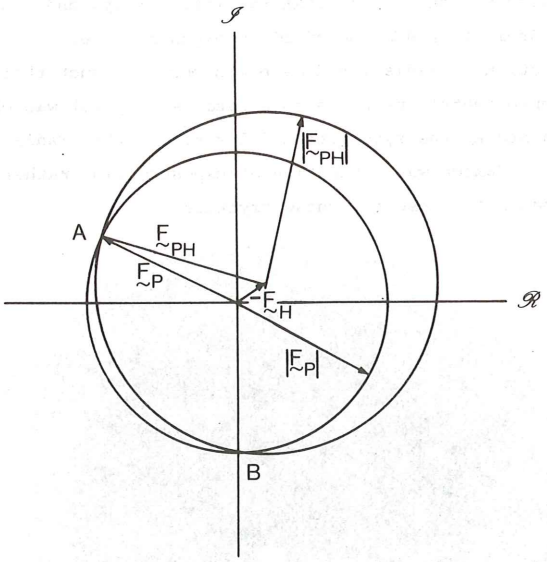


Fig. 2

The atomic scattering factor can be written:

$$f = f_o + f'(\omega) + if''(\omega)$$

where f' and f" are respectively the real and imaginary parts of the anomalous component, ω is the frequency of the incident wave.

For crystal structures containing only light atoms (C,N,O,H) f' and f" are negligible at x-ray wavelengths and

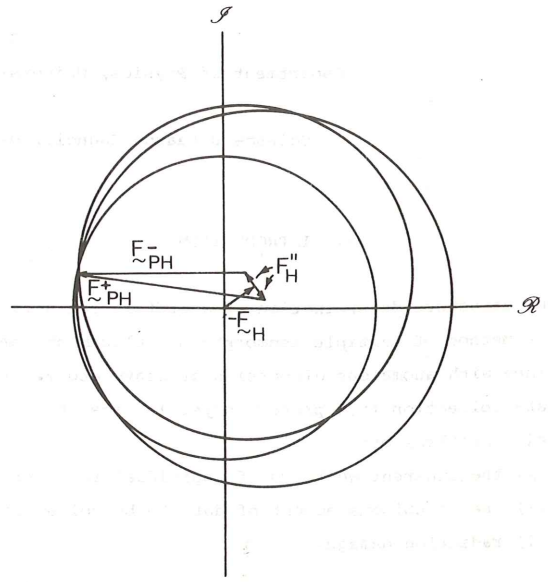


Fig. 3

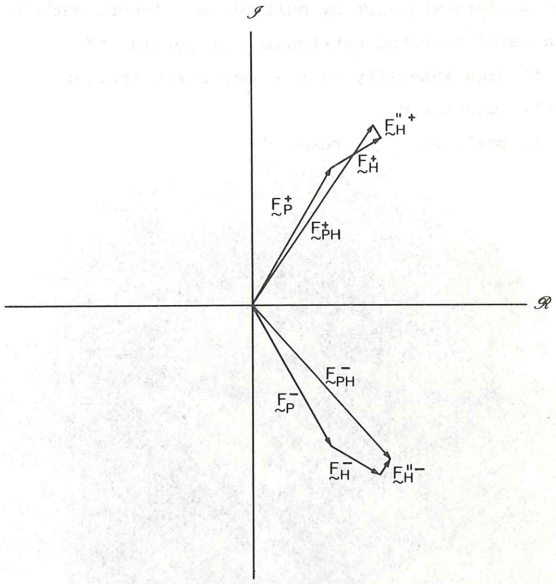


Fig. 4

$$|F^{(+)}_{\underline{P}}| = |F^{(-)}_{\underline{P}}| \qquad \text{(Friedel's Law)}$$

However, when an anomalously scattering heavy atom is added, yielding an N + 1 atom structure:

$$F_{\underline{PH}} = \sum_{j=1}^{N} f_j \exp(2\pi i \underline{h} \cdot \underline{x}_j)$$

$$+ (f_{N+1} + f'_{N+1} + f''_{N+1}) \exp 2\pi i \underline{h} \cdot \underline{x}_{N+1}$$

and then

$$|F^{(+)}_{PH}| \neq |F^{(-)}_{PH}| \qquad \text{(fig.4)}$$

The anomalous difference $|F^{(+)}_{PH}| - |F^{(-)}_{PH}|$ can be utilised along with the isomorphous information to solve the phase i.e. (in standard notation) the equations

$$\cos(\alpha_H - \alpha_{PH}) = (F^2_{PH} - F^2_P + F^2_H)/2F_{PH}F_H$$

and

$$\cos(\alpha_H'' - \alpha_{PH}) = \frac{(F^{(+)2}_{PH} - F^{(-)2}_{PH})}{4F_{PH}F_H''}$$

yield the unique value of α_{PH} and the phase α_P of the protein reflection is given by

$$\alpha_P = \tan^{-1}\frac{(F_{PH}\sin\alpha_{PH} - F_H\sin\alpha_H)}{(F_{PH}\cos\alpha_{PH} - F_H\cos\alpha_H)}$$

(from Kartha[2]). This method is known as single isomorphous replacement with anomalous scattering (SIRAS).

Using the calculations of Crick and Magdoff[3] an estimate can be made of the average fractional change in intensity as a consequence of a change f" in the scattering factor of the anomalously scattering heavy atom.

The fractional change in intensity for a structure with N normally scattering atoms with scattering factor f_o is given by

$$\frac{\Delta}{I} = \left(\frac{2}{N}\right)^{1/2}\frac{f''}{f_o} \qquad \text{(for an acentric reflection)}$$

For example for the enzyme 6PGDH with an average f_o = 7, and containing one fully occupied platinum heavy atom site (f" ≈ 7, at 1.54 Å) for the $K_2Pt(CN)_4.3H_2O$ derivative (Adams et al[1]) in an asymmetric unit of 50,000 molecular weight, the fractional change is 2.4% giving an intensity difference between Friedel pairs of 4.8%.

In this case an accuracy of at least this is required in measurement. The limit of accuracy for intensity measurements made with photographic film on the Arndt-Wonacott rotation camera as indicated by a reliability index R* is approximately 3% on F (6% on I) (Arndt et al[4]).

The larger f", the larger the intensity difference between Friedel equivalents and the more accurate will be the determination of the phase. The magnitude of f" (and f') varies with wavelength λ, and by careful choice of λ, f" can be optimised. For a K absorption edge (fig.5) f' is approximately symmetrical about λ_e, the wavelength of the edge, whereas f" is ≈ 0 for $\lambda > \lambda_e$. With L edges, however, f" does not vanish on the long wavelength side (fig.5).

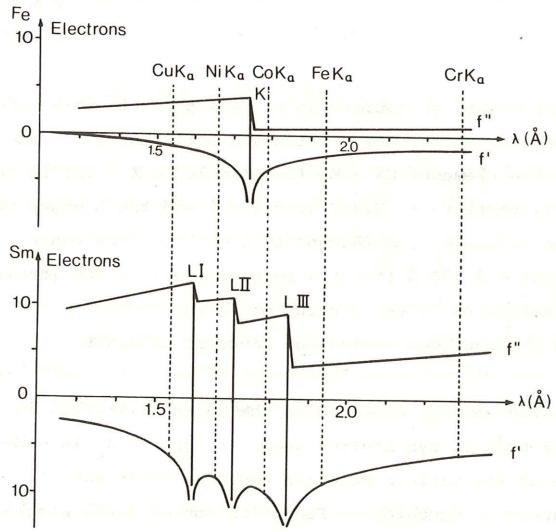


Fig. 5

In a recent study of the L_{III} edge of Cs(f_o = 38 at sin θ/λ = 0.3 Å^{-1}) (Phillips et al[5]) at λ = 2.473Å values of f' = -27.1 and f" = 5.9 and at λ = 2.470 Å, f' = -16.1 and f" = 11.1 were observed. With such a large value of f', $f_{real} = f_o + f'$ = 10.9 at 2.473 Å with the effect that the atom is barely distinguishable from the surrounding C,N,O atoms. It has to some extent been contrasted out.

The maximum value of f" = 11.1 which is 20% of f_o(= 55e$^-$). Since values of f' and f" are not available for Pt at the L edge it has to be assumed that such a fractional change in f_o of f" would be reproduced with the L_{III} edge of Pt(λ_e = 1.07239 Å). Then f" becomes 16e$^-$ from 7e$^-$, and Δ/I for 6PGDH = 5.5% establishing these changes at a significance level above the errors of the screenless rotation photographic method.

$$^*R = \left[\sum_i (F_{hi} - F_h)^2/n^{1/2}\right]/\left[\sum_i F_{hi}/n\right]$$

TABLE 1

Machine specification and parameters relevant to the spectra of various synchrotron radiation sources.

Machine (location)	E(GeV)	I(mA)	R_{meters}	ε_c keV	λ_c	Remarks
SRS (Daresbury U.K.)*	2.0	500-1000	5.55	3.2	3.87	dedicated
			1.33	13.3	0.93	from 5T wiggler
DCI (Orsay)*	1.8	500	3.8	3.4	3.65	very similar λ_c to Daresbury SRS
DORIS (Hamburg)+	4	100	12.1	11.7	1.06	optimal parameters for hard x-rays.

*from Winick ref.(6)

+H. Stuhrmann, ref.(7)

This method of optimising f" is applicable to a wide range of atomic number elements utilising the K edges of the elements Ca → Mo (i.e Z = 20 to Z = 42) in the wavelength range 3.070 to 0.620 Å and the L edges of the elements Sb-U (Z = 51 to Z = 92) in the range 2.633 - 0.570 Å (for L_I) respectively. At the longer wavelengths, x-ray absorption in the Be window and in the specimen limits the range of elements.

At the shorter wavelengths the major limitation is the type of synchrotron used. In Table 1 λ_c is compared for various machines and only DORIS and the Daresbury Synchrotron Radiation Source (SRS) wiggler line has a spectrum for which anomalous scattering experiments for elements at the short λ end of the K and L edge range are feasible.

The method of optimised f" SIRAS is useful for protein structure studies where only a single <u>isomorphous</u> derivative can be obtained and is especially suitable for a large protein where there is little or no centric data. However, changes in f' with wavelength are specially suited to studies of metalloproteins or proteins for which only a non-isomorphous derivative can be prepared. It is possible to consider the use alone of anomalous dispersion effects without the necessity for preparing an isomorphous derivative.

The use of f' and f" for phase determination is simple to visualise:

(i) measurement for FPH, only, at 3 different wavelengths (with no Friedel information) is analogous to the measurement of "native" and two derivatives because of the variation of f' with λ.

(ii) measurement of FPH^+ and FPH^- at two non-matching wavelengths on either side of an edge, for which f' is different, would give a unique determination of the phase

e.g. for a K edge

for $\lambda \gtrsim \lambda_e$, f" ≈ 0 and $FPH_{\lambda 1}{}^+ \simeq FPH_{\lambda 1}{}^-$

for $\lambda \lesssim \lambda_e$, f" ≠ 0 and $F_{PH\lambda 2}{}^+ \neq F_{PH\lambda 2}{}^-$

and $F_{PH\lambda 1} \neq F_{PH\lambda 2}$

Here, $FPH_{\lambda 1}{}^+$, $FPH_{PH\lambda 2}{}^+$, $F_{PH\lambda 2}{}^-$ can be used in a way analogous to SIRAS as described above as a necessary and sufficient basis for phase determination.

In both cases (i) and (ii) if a sequence of measurements can be made off one crystal and with such small changes of λ, the path of the diffracted beam through the crystal is the same then this means a concomitant reduction in errors compared to the classical techniques of isomorphous replacement.

Hoppe and Jakubowski[8] pioneered the use alone of anomalous dispersion effects and by means of the two wavelength method obtained the structure of the Fe protein erythrocruorin which compared favourably with the published structure of Huber et al[9]. The experiment was limited to two wavelengths, depending as it did on conventional Ni K_α (λ = 1.659 Å) and Co K_α (1.790 Å) tubes.

With the advent of synchrotron radiation a whole sequence of measurements can be made at different wavelengths about an absorption edge (Phillips et

al[10,11]). It is not yet clear which is the most suitable combination of measurements to make in order to obtain the best solution of a structure for the most economical use of x-ray beam time.

3. PROPOSED EXPERIMENTAL ARRANGEMENT AT THE DARESBURY SRS FOR PROTEIN CRYSTAL STUDIES

In terms of the spectral profile of the Daresbury SRS, currently under construction, anomalous scattering experiments of elements with absorption edges at wavelengths < 1.0 Å require use of the wiggler x-ray beam line (λ_c = 0.932 Å). At longer wavelengths anomalous dispersion phasing methods can be explored on the first x-ray beam line. The full use of absorption edges in this way has specific needs

(i) a monochromator resolution $\Delta\lambda/\lambda < 10^{-3}$ (Phillips et al[5]).

(ii) a fixed beam position and direction as λ changes, for feasible running of such an experiment.

These needs are best satisfied by use of a channel cut monochromator which also suits EXAFS experiments.

These requirements contrast with those of experiments depending on high intensity. In particular, the finer the monochromator resolution used to explore an absorption edge the lower the intensity striking the sample. Also native protein diffraction measurements can be conveniently measured at a single wavelength (e.g. 1.5 Å) for which no problems of beam movement arise.

3.1 Estimated X-ray Flux at the Crystal

3.1.1 High flux line

The flux for the proposed SRS set-up can be based on the Orsay camera. DCI routinely works at 1.72 GeV, 120 mA, λ_c = 3.8 Å with approximately 1.4 mrad horizontal radiation. A bent Ge crystal 7 cm long is used at DCI on the protein crystallographic facility with an object distance of 15.5 m and an image distance of 1.7 m to focus the source size of 5 x 1 mm^2 (at 2σ) to a line focus of width 0.56 mm (Lemonnier et al[12]) The vertical extension is limited by a slit to 4.5 mm at the focus and the measured flux is 6×10^{10} hν/s.

The Daresbury SRS and DCI have very similar source flux and wavelength profiles but different source sizes. The SRS source size is 13.7 x 0.43 mm^2 at 2σ. When comparing the intensity from a similar monochromator arrangement, a correction has to be made to account for the different source sizes of the SRS compared to DCI.

At DCI the horizontal source is demagnified by a factor of 1.7/15.5 from 5 mm to 0.5 mm. The vertical size at the sample, for a 0.4 mm rad vertical divergence = (17.2 x 0.4 + 1) = 7.88 mm. Similar calculations for the SRS, using for ease of comparison the same focal distances, show that in the sample plane the horizontal line width = 13.7 x 1.7/15.5 = 1.5 mm and the length of the line = (17.2 x 0.4 + 0.43) = 7.31 mm. Therefore, the SRS flux is given by

$$6 \times 10^{10} \times \frac{200}{120} \quad \frac{0.56}{1.5} \quad \frac{7.88}{7.31} = 6.0 \times 10^{10} \text{ hν/s}$$

an intensity which is the same as at DCI. The larger circulating current compensating for the larger source size of the SRS compared to DCI.

An obvious way to improve on this intensity is to introduce vertical focusing, which would also improve spot resolution at the detector. Since the SRS vertical size is 0.43 mm (at 2σ) 1:1 focusing is sufficient.

3.1.2 Fine spectral resolution, anomalous scattering experiments.

A channel cut Si(111) monochromator, which is being set up at the Daresbury SRS for EXAFS work, has a specification which matches the needs of the optimised anomalous dispersion work ideally.

At 1.5 Å, the flux delivered at the sample is 3×10^8 hν/s/mA/mrad horiz./eV which for 200 mA circulating current and 2 mrad horizontal radiation gives a flux = 1.2×10^{11} hν/s in a beam of size 13.7 x 7.31 mm^2 with 1:1 horizontal focusing. A sample of size 0.3 x 0.3 mm^2 intercepts a flux = 1×10^9 hν/s/eV. Again use of vertical focusing would improve the flux intercepted by the sample considerably.

3.2 Summary of SRS Facilities

When the Daresbury SRS is commissioned in 1980 the curved Ge monochromator described above will be used in conjunction with an Arndt-Wonacott rotation camera with data collected on photographic film. The same camera, or alternatively a precession camera,

can be used for fine spectral resolution anomalous dispersion work if coupled to the EXAFS monochromator.

4. CONCLUSIONS

Synchrotron radiation can be of considerable use to protein crystallography and the advantages to the user of a dedicated source like the Daresbury SRS cannot be over-emphasised. High intensities enable accurate high resolution data to be collected rapidly and the tunability allows use of anomalous dispersion techniques for phase determination of proteins.

ACKNOWLEDGEMENTS

Thanks are due to J.Worgan for supplying the calculations on the proposed monochromator arrangement for protein crystallography at the Daresbury SRS and Dr. R.Fourme for use of the Orsay facility to enable the photographs in fig.1 to be taken. Support from SRC for a research studentship and the MRC for a post-doctoral fellowship while a member of the Laboratory of Molecular Biophysics, University of Oxford, Head, Professor D.C.Phillips, FRS is acknowledged. Grateful thanks are also due to Dr. M.J.Adams for discussions and encouragement and also, Dr. J.C.Phillips for discussions on the establishment of separated multiple wavelength and high flux experiments (section 3) and for comments on the manuscript.

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