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Unique Phase Determination on the Basis of Bijvoet Ratios by Means of Resonant Neutron Scattering

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A new two-wavelength Bijvoet-pair method is proposed, which allows unique experimental determination of anomalous phases without absorption correction and scaling of the Bragg intensities, if the resonant atom structure is known. The method consists of measuring Bijvoet ratios at two wavelengths λ_1 and λ_2, for which the real and imaginary dispersion terms in the anomalous scattering amplitude fulfil the conditions: $b'_A(\lambda_1) = -b'_A(\lambda_2)$ and $b''_A(\lambda_1) = b''_A(\lambda_2)$. These conditions can be fulfilled with resonant neutron scattering by nuclei like ^{113}Cd, ^{149}Sm or ^{157}Gd, but not with resonant X-ray or Mössbauer γ-ray scattering. A first application of the method to experimental phase determination for a small structure, Cd-histidine, is described.

Introduction

The use of two-wavelength Bijvoet-pair methods for unique phase determination for protein structures by means of anomalous X-ray scattering was proposed by Herzenberg & Lau (1967) and Srinivasan & Chacko (1970). These techniques are based on Bijvoet *differences* and therefore require absolute structure factors. Unangst, Müller, Müller & Keinert (1967) proposed a method based on Bijvoet *ratios* rather than differences, which avoids the necessity of absolute scaling. This method can be applied, if the approximation

$$\left|\frac{2}{R}\, b^0_A b''_A \sin\tilde{\alpha} + b^0_A b'_A \cos\tilde{\alpha}\right| \gg (b'_A)^2 + (b''_A)^2$$

is valid. The angle $\tilde{\alpha}$ is defined in Fig. 1. b^0_A and b'_A, b''_A are the nonresonant and resonant terms, respectively, of the scattering amplitude of the reference scatterers,

$$b_A = (b^0_A + b'_A) + ib''_A.$$

R is the Bijvoet ratio for the reflection pair $H, \bar{H}$,

$$R = \frac{D}{M} = \frac{|F(H)|^2 - |F(\bar{H})|^2}{(|F(H)|^2 + |F(\bar{H})|^2)/2}.$$

In the case of resonant neutron scattering by nuclei such as ^{113}Cd, ^{149}Sm or ^{157}Gd (Ramaseshan, 1966), and Mössbauer γ-ray scattering by ^{57}Fe (Black & Duerdoth, 1964; Parak, Mössbauer & Hoppe, 1970), the dispersive terms in the scattering length may greatly dominate the nonresonant term. The above-described approximation is therefore not valid for these techniques.

Two-wavelength Bijvoet ratio (2WL-BR) method

A method for unique phase determination, which in principle does not involve any approximation, can be formulated for resonant neutron scattering, if the positions of the anomalous scatterers in the unit cell are known. The dependence of the resonant scattering lengths b'_A and b''_A on the wavelength is well described by the single-level Breit–Wigner formula (Smith & Peterson, 1964). The width of thermal neutron resonances ($\Gamma \sim 0{\cdot}1$ eV) is very broad compared with the width of the energy distribution in the incident beam. It is therefore possible to choose wavelength pairs λ_1, λ_2, for which the following conditions are fulfilled simultaneously:

$$b'_A(\lambda_1) = -b'_A(\lambda_2) \quad \text{and} \quad b''_A(\lambda_1) = b''_A(\lambda_2). \tag{1}$$

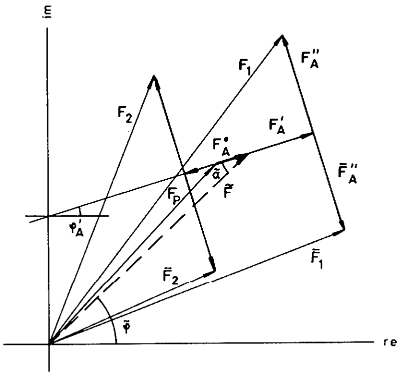

Fig. 1. Argand diagram illustrating the principle of the 2WL-BR method of experimental phase determination. F°_A is the nonresonant, F'_A and F''_A are the resonant contributions of the reference scatterers to the total structure factors $F = F(H)$ and $\bar{F} = F(\bar{H})$. F_P is the structure factor contribution from the rest of the unit cell.

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Fig. 2 shows an example for such a choice of wavelengths. The conditions (1) cannot be fulfilled if resonant X-ray (Hoppe & Jakubowski, 1975) or Mössbauer γ-ray scattering techniques are employed for phase determination.

The difference angle, $\tilde{\alpha}(H) = \tilde{\varphi}(H) - \varphi'_A(H)$, and hence the phase angle $\tilde{\varphi}(H)$, of the structure factor $\tilde{F}(H)$, which includes the nonresonant contributions from all scatterers in the unit cell (see Fig. 1), can be calculated from the following equations, if the conditions (1) are fulfilled:

$$\begin{aligned} D_1 = D_2 &= 4|\tilde{F}|\,|F''_A|\sin\tilde{\alpha} \\ M_i &= |\tilde{F}|^2 + |F'_A|^2 + |F''_A|^2 \\ &\quad + (-1)^i 2|\tilde{F}|\,|F'_A|\cos\tilde{\alpha} \quad (i = 1, 2), \end{aligned} \tag{2}$$

where D_i is the Bijvoet difference, M_i the average intensity of the reflection pair H, $\bar{H}$ at the wavelength λ_i. F'_A and F''_A denote the structure factor contributions from the resonant scatterers. One may easily derive from these equations an expression for $\tan\tilde{\alpha}$, which involves only Bijvoet ratios:

$$\tan\tilde{\alpha} = \frac{|b'_A|}{|b''_A|}\left(\frac{1}{|R_1|} - \frac{1}{|R_2|}\right)^{-1} \operatorname{sign}(D_i). \tag{3}$$

Equation (3) leads to a unique solution for the angle $\tilde{\alpha}$, if $\tilde{\alpha} \neq 0$ or π. This 2WL-BR method offers the following systematic advantages for experimental phase determination compared with techniques involving Bijvoet differences.

(i) The Bijvoet ratio is to very good approximation free of absorption effects, if *direct* Bijvoet pairs are measured. The ratio will in general depend on extinction and thermal diffuse scattering. It may, however, because of the small average intensity of protein reflections, be assumed that these effects are negligible. Scaling of the experimental structure factors is therefore not necessary.

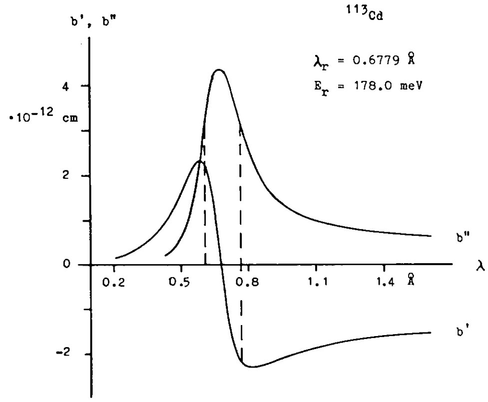

Fig. 2. Breit-Wigner single-level values of the dispersion terms b'_A and b''_A for the 178 meV neutron resonance of ^{113}Cd. The scattering length of the reference scatterer is $b_A = b^{\circ}_A + b'_A + ib''_A$. The dashed lines indicate a wavelength pair (λ = 0·61/0·77 Å), for which the conditions (1) are fulfilled.

(ii) Equation (3) does not contain resonant structure factor contributions but only the dispersion terms in the scattering length, which can be calculated from the Breit-Wigner formula. The occupancy and temperature factor of the resonant scatterer do not enter into (3).

(iii) Only relative structure factors are required. The determination of integrated intensities can therefore be replaced by measuring peak count rates.

(iv) The total phase error in α contains only contributions from counting statistics and from errors in the background correction. This facilitates a reliable estimation of the phase error.

Optimum choice of wavelengths

The conditions (1) can be fulfilled, if the two wavelengths are chosen on either side of a resonance. A criterion for the optimum choice of the wavelength pair is in the following derived from the wavelength dependence of the average phase error.

We assume that the average intensities M_1 and M_2 are measured to the same relative accuracy $\Delta I/I$. Developing (3) for small errors $\Delta\tilde{\alpha}$ in the difference angle $\tilde{\alpha}$ leads to the expression

$$\begin{aligned} \Delta\tilde{\alpha} &\sim \frac{|\cos\tilde{\alpha}|}{|S_1 - S_2|}\,\frac{\Delta I}{I}\,(M_1^2 + M_2^2)^{1/2} \\ &\quad \times \left(\left|\frac{b'_A}{b''_A}\right|^2 \cos^2\tilde{\alpha} + \sin^2\tilde{\alpha}\right)^{1/2}, \end{aligned} \tag{4}$$

where

$$S_i = M_i - |F'_A|^2 - |F''_A|^2.$$

From (2)

$$|S_1 - S_2| = 4|\tilde{F}|\,|F'_A|\,|\cos\tilde{\alpha}|$$

and, for small anomalous effects,

$$(M_1^2 + M_2^2)^{1/2} \sim \sqrt{2}\,|\tilde{F}|^2;$$

hence

$$\Delta\tilde{\alpha} \sim \frac{\sqrt{2}}{4}\,\frac{\Delta I}{I}\,\frac{|\tilde{F}|}{|F'_A|}\left(\left|\frac{b'_A}{b''_A}\right|^2 \cos^2\tilde{\alpha} + \sin^2\tilde{\alpha}\right)^{1/2}.$$

If one neglects the correlation between $|\tilde{F}|$, $|F'_A|$ and $\tilde{\alpha}$, and inserts the average values corresponding to the Wilson theory,

$$\langle|\tilde{F}|\rangle = \left(\frac{\pi}{6} N_P \langle b_P\rangle^2\right)^{1/2}$$

$$\langle|F'_A|\rangle = \left(\frac{\pi}{4} N_A (b'_A)^2\right)^{1/2},$$

Table 1. *Optimum wavelengths for an application of the 2WL-BR method for the practically most important neutron resonances*

The λ_{opt} have been calculated from equation (5). In the case of ^{157}Gd, λ_{opt} has been chosen within the wavelength range $\lambda \leq 3$ Å (see the remark made in the text).

	λ_{opt} (Å)	$\lvert b'_A \rvert$ (10^{-12} cm)	b''_A (10^{-12} cm)
^{113}Cd	0·61/0·77	2·17	3·08
^{149}Sm	0·82/1·04	2·86	4·04
^{157}Gd	1·23/3·00	2·52	5·99

where N_P and N_A are the numbers of nonresonant and resonant scatterers, respectively, and $\langle b_P \rangle$ is the average scattering amplitude of the nonresonant scatterers in the unit cell, one obtains:

$$\frac{|\tilde{F}|}{|F'_A(\lambda)|}\left(\left|\frac{b'_A(\lambda)}{b''_A(\lambda)}\right|^2 \cos^2\tilde{\alpha} + \sin^2\tilde{\alpha}\right)^{1/2} \propto \left(\frac{N_P}{N_A}\right)^{1/2} \frac{\langle b_P \rangle}{|b'_A(\lambda)|}\left(\left|\frac{b'_A(\lambda)}{b''_A(\lambda)}\right|^2 + 1\right)^{1/2}.$$

Test calculations on the basis of the structural model of Cd-histidine showed that this relation is to good approximation valid within the whole wavelength range of the resonance. The average phase error is then proportional to

$$\langle \Delta\tilde{\alpha} \rangle \propto \frac{\Delta I}{I} \langle b_P \rangle \cdot \left(\frac{N_P}{N_A}\right)^{1/2} \varepsilon(\lambda). \tag{5}$$

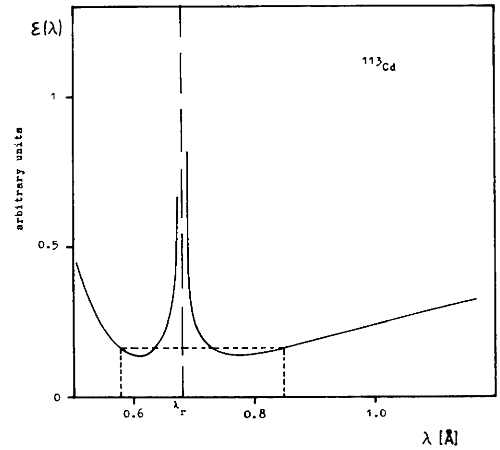

Fig. 3. Wavelength-dependent factor $\varepsilon(\lambda)$ in the average phase error $\langle \Delta\tilde{\alpha} \rangle$ described by equation (5). $\varepsilon(\lambda)$ is displayed in relative units for the example of the ^{113}Cd resonance. The dashed lines indicate a wavelength pair (λ = 0·58/0·85 Å), for which the conditions (1) are fulfilled.

The wavelength dependence of $\langle \Delta\tilde{\alpha} \rangle$ is contained in the factor ε,

$$\varepsilon = \frac{[(b'_A)^2 + (b''_A)^2]^{1/2}}{|b'_A b''_A|}.$$

Inserting the well-known Breit–Wigner expressions for the dispersion terms b'_A and b''_A (Smith & Peterson, 1964), one derives easily that $\varepsilon(\lambda)$ goes through minima at neutron wavelengths corresponding to energies E_i,

$$|E_i - E_r| = \frac{1}{2\sqrt{2}}\Gamma \quad (i = 1, 2).$$

E_r is the resonance energy, Γ is the total width of the resonance. Fig. 3 shows $\varepsilon(\lambda)$ for the example of the ^{113}Cd resonance. The resulting optimum wavelengths are listed in Table 1 for the practically most important neutron resonances. A solution at negative energy is obtained in the case of the 31·4 meV resonance of ^{157}Gd. The wavelength pair indicated in the table has been chosen within the range $\lambda \leq 3$ Å in order to permit data collection to very high resolution ($\lambda/\sin\theta > 1{\cdot}5$ Å). The optimum choice of the wavelengths is in addition influenced by the experimental conditions; this is discussed elsewhere (Bartunik, 1978).

Application to phase determination for Cd-histidine

The 2WL-BR method has been tested in a first application to a small structure, Cd-histidine, using resonant neutron scattering by ^{113}Cd in its natural isotopic abundance (12·26%).

Tetragonal bis(L-histidinato)cadmium dihydrate, $Cd(C_6H_8N_3O_2)_2.2H_2O$, crystallizes in space group $P4_32_12$ with lattice parameters $a = b = 7{\cdot}397$, $c = 30{\cdot}53$ Å, $Z = 4$. The X-ray structure was determined by Candlin & Harding (1967). The complete structure including the hydrogen positions was on the basis of neutron data refined to an $R(F^2)$ factor of 6·9% (Fuess & Bartunik, 1976).

Direct Bijvoet pairs were measured for a set of 17 reflections corresponding to d spacings between 1·1 and 3·3 Å at two wavelengths λ_1 = 0·590, λ_2 = 0·818 Å on either side of the ^{113}Cd resonance (λ_r = 0·678 Å). Both the average Bijvoet ratio ($\langle R \rangle = 28\%$) and the average structure factor of the reflections were in agreement with the corresponding average values calculated for the complete structure. The measurements were carried out on a four-circle diffractometer (D9) at the hot source of the HFR Grenoble. A plate-like crystal, 1·2 × 1·2 × 0·7 mm, was used. The linear absorption coefficient μ, was 9·0 cm^{-1} at 0·590 Å, and 12·4 cm^{-1} at 0·818 Å.

Phases were derived from (3) on the basis of integrated intensities (ω-scan, 50 steps per reflection) and of peak count rates. The intensities were in both

Table 2. *Results of phase determination for* Cd-*histidine with the* 2*WL-BR method*

The experimental phase angles $\tilde{\varphi}_{\text{exp}}$ given in column I have been derived from integrated intensities, in column II from peak count rates. $\Delta\tilde{\varphi} = |\tilde{\varphi}_{\text{exp}} - \tilde{\varphi}_{\text{th}}|$ is the difference between $\tilde{\varphi}_{\text{exp}}$ and the 'theoretical' value $\tilde{\varphi}_{\text{th}}$ (column III) calculated from the structural model.

	I			II		III
	Phases derived from					
	integrated intensities			peak count rates		
$h\,k\,l$	$\tilde{\varphi}_{\text{exp}}$	$\sigma(\tilde{\varphi}_{\text{exp}})$ (°)	$\Delta\tilde{\varphi}$	$\tilde{\varphi}_{\text{exp}}$ (°)	$\Delta\tilde{\varphi}$	$\tilde{\varphi}_{\text{th}}$ (°)
1 2 1	92·27	6·9	9·96	86·77	15·46	102·23
1 2 2	−28·19	26·6	52·64	−11·40	69·43	−80·83
2 1 2	112·81	34·7	31·98	10·59	70·24	80·83
1 2 3	−155·48	27·1	15·81	−157·70	13·59	−171·29
2 1 3	−6·62	11·9	2·09	−39·85	31·14	−8·71
1 2 4	−98·78	13·6	32·75	−118·46	13·07	−131·53
2 1 4	77·01	16·7	54·52	147·29	15·76	131·53
1 2 9	−29·66	5·3	19·08	−39·04	9·70	−48·74
2 1 9	−153·73	19·7	22·47	−153·90	22·64	−131·26
1,2,16	86·57	12·8	2·41	127·00	38·02	88·98
2,1,16	−128·09	33·7	39·11	−47·10	41·88	−88·98
1,3,15	140·14	24·6	3·84	110·39	25·91	136·30
3,1,15	65·26	17·2	21·56	85·26	41·56	43·70
1 6 4	60·81	16·8	6·05	54·39	12·47	66·86
6 1 4	−94·72	8·8	27·86	−83·48	16·62	−66·86
1 6 8	123·73	17·7	1·04	98·86	23·83	122·69
6 1 8	−79·13	13·0	43·56	−102·67	20·02	−122·69

cases corrected for isotropic background due to incoherent hydrogen scattering. They were used without any further correction or scaling. The results are compiled in Table 2. The average phase error relative to phases calculated from the structural model is $\langle\Delta\tilde{\varphi}\rangle = |\tilde{\varphi}_{\text{exp}} - \tilde{\varphi}_{\text{th}}| = 22{\cdot}7°$ for phases derived from integrated intensities, and 28·3° for phases from peak count rates. This difference in the average phase error is essentially due to different counting statistics.

The total phase error $\sigma(\tilde{\varphi}_{\text{exp}})$ is essentially given by the error on the experimentally determined difference phase angle $\tilde{\alpha}$. $\sigma(\tilde{\alpha})$ has been estimated from (4). The average uncertainty in the 'theoretical' phase angles $\tilde{\varphi}_{\text{th}}$ is estimated to be $\sigma(\tilde{\varphi}_{\text{th}}) \sim 5°$. This uncertainty has to be added to the predicted phase error $\sigma(\tilde{\varphi}_{\text{exp}})$ for a comparison with the observed differences $\Delta\tilde{\varphi}$. One finds that

$$\Delta\tilde{\varphi} < 3[\sigma^2(\tilde{\varphi}_{\text{exp}}) + \sigma^2(\tilde{\varphi}_{\text{th}})]^{1/2}$$

for all reflections. This means that individual phase errors could be estimated for the anomalous phases.

Conclusion

The technique proposed here for phase determination by means of resonant neutron scattering leads to unique phase solution independent of absorption correction and scaling. It should therefore be of considerable interest for protein structure analysis. The present application demonstrates further that peak count rates could be used instead of integrated intensities. The time needed for data collection can thus be reduced by one order of magnitude.

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