9.1. Introduction

Anomalous scattering is not a new subject. It was already introduced in Chapter 7. There, you learned that anomalous scattering by an atom is due to the fact that its electrons cannot be regarded as completely free electrons. This effect depends on the wavelength, but it is, in general stronger, for the heavier atoms than for the light atoms in the periodic system. If heavy atoms are present in a protein structure, the consequence of their anomalous scattering is that the intensities of a reflection $h k l$ and its Bijvoet mate $\bar{h} \bar{k} \bar{l}$ are no longer equal. In Chapter 7, this effect was used in combination with the isomorphous replacement differences in the search for the heavy atom positions and in the refinement of these positions. In this chapter it will be shown how anomalous scattering information can help to determine the phase angle of the protein reflections and the absolute configuration of the protein structure. Moreover, it will be discussed how anomalous scattering is exploited for protein phase angle determination by the multiple-wavelength anomalous diffraction (MAD) method and by single-wavelength anomalous diffraction (SAD).

9.2. Protein Phase Angle Determination with Anomalous Scattering

In principle, the anomalous scattering by heavy atoms contributes to the determination of the protein phase angles as much as the isomorphous replacement does. This can best be explained in Figure 9.1. Three circles are drawn in Figure 9.1, with radii $F_p$, $F_{PH}(+)$, and $F_{PH}(-)$; the (+) and the (−) indicate a Bijvoet pair of reflections. The $F_p$ circle has its center at $O$. For the $F_{PH}(+)$ circle, the
Figure 9.1. The Harker diagram for protein phase angle determination by anomalous scattering. \(|F_P|\) is the structure factor amplitude for the native protein and \(|F_{PH}(+)\) and \(|F_{PH}(-)\) are those for the Friedel mates of the heavy atom derivative. The contribution to the structure factor by the heavy atom is \(F_H(+)\) for one member of the Friedel pair and \(F_H(-)\) for the other member. These two structure factors are not symmetric with respect to the horizontal axis because of an anomalous component. The positions of the intersection points \(\alpha_1\) and \(\alpha_1'\) do have a position symmetric with respect to the horizontal axis because the structure factor of the native protein has no anomalous component.

center is at the end of the vector \(-F_H(+)\), and for the \(F_{PH}(-)\) circle, it is at the end of the vector \(-F_H(-)\). The two intersections of the \(F_P\) and \(F_{PH}(+)\) circles at \(\alpha_1\) and \(\alpha_2\) indicate two possible protein phase angles. Two other possibilities are found at the two intersections of the circles \(F_P\) and \(F_{PH}(-)\): \(\alpha_1'\) and \(\alpha_2'\). Because the reflections \((h\,k\,l)\) and \((\bar{h}\,\bar{k}\,\bar{l})\) of the native protein crystal have opposite phase angles (Section 4.11 of Chapter 4), the correct choice is for the phase angles \(\alpha_1\) for \((h\,k\,l)\) and \(\alpha_1'\) for \((\bar{h}\,\bar{k}\,\bar{l})\). This is illustrated in a simpler way in Figure 9.2. Here, the vector \(-F_H(-)\) is drawn with the opposite phase angle (mirror image with respect to the horizontal axis). Now the correct phase angle is found at the intersection of the three circles \(F_P\), \(F_{PH}(+)\), and \(F_{PH}(-)\), assuming that the data are error-free. The conclusion is that, in principle, the protein phase angle problem can be solved with one isomorphous heavy atom derivative if anomalous scattering is incorporated (SIRAS).