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PROBING THE IRON-SULPHUR PROTEINS: A BIOCHEMIST'S VIEW OF SPECTROSCOPIC METHODS

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1. Introduction. — Biochemists, like other biologists, are studying structures that have been refined over the course of evolution to become constituents of the living world. In this case, the structures are molecules, such as enzymes and nucleic acids. They are too small to observe directly so it is necessary to use indirect methods such as X-ray diffraction and spectroscopy.

The importance of X-ray diffraction in visualizing the structures of macromolecules, particularly enzymes and transfer RNA molecules, cannot be overestimated. However the size of the molecules that can be studied in this way is limited, and they must be in the pure state. Moreover, even when the complete structure is presented, it is often not immediately obvious how the thing works. The action of an enzyme or electron transfer protein, for example, depends on electronic rearrangements that take place within a very restricted part of the huge molecule, the active site. One of the purposes of spectroscopic investigations is to elucidate the processes taking place at the active site.

The UV/visible spectrophotometer, the workhorse of biochemical investigations, illustrates many of the features of a desirable spectroscopic technique. It is applicable to a wide range of biological materials, and the high degree of resolution in an absorption spectrum means that a number of compounds may be identified in the same sample without the need for complete purification. The measurements are sensitive and quantitative. Since the method is non-destructive, chemical changes can be followed continuously in complex systems. The applications of the techniques

Abstract. — Biochemists routinely use UV/visible spectrophotometry as a means of identifying and measuring the quantity of many biological compounds. When a new type of compound is isolated however, the method is often unhelpful in determining its chemical nature, and other spectroscopic methods must be used. The iron-sulphur proteins have recently been recognised as major components of bioenergetic systems such as respiration and photosynthesis. They represent a totally new type of biological compound. The simplest types of iron-sulphur proteins, the ferredoxins, have been investigated by a wide range of spectroscopic methods, including magnetic resonance and Mössbauer spectroscopy. Such methods involve problems in the preparation of concentrated, pure samples, and the work requires a close cooperation between the preparative biochemist and the spectroscopist. The results of application of different spectroscopic techniques on the eight-iron ferredoxin from Clostridium pasteurianum are described, and the results are compared with those from a chemical analog of the iron-sulphur cluster. The protein clearly has an influence on the properties of the iron-sulphur cluster, as shown by modifications to its oxidation-reduction potential and reactivity. The applications of Mössbauer spectroscopy are being extended to the more complex iron-sulphur proteins such as nitrogenase, and to the photochemical reactions of photosynthesis.
are still being extended, for example, in the development of rapid-reaction spectrophotometers, which can now operate on the picosecond timescale [1, 2]. Spectrophotometry is a very useful practical tool in biochemistry, but it is often unhelpful in defining the fundamental chemistry of a compound. If we isolate a totally new type of compound, the UV/visible absorption spectrum would be unlikely to tell us much about its structure. Spectrophotometry is therefore a fingerprint technique, which relies on our recognising spectra as similar to others we have seen before. However this is not such a disadvantage to the method as might at first appear. Living systems contain an enormously varied and apparently eccentric collection of chemical compounds, but most of them fall into a relatively small number of types. For example, the enormous variety of plant pigments are mostly variants of a few organic structures. Often the biochemist can recognize the spectrum as belonging to one of these types of structures, and even determine to which sub-group it belongs by the exact position of the absorption peaks. Therefore, once the structures of a few of these compounds have been determined by other means, many similar compounds can then be studied by the spectrophotometric method.

Not all biochemical compounds absorb in the UV/visible region however, and for those that do, the spectra of some are much more easily recognizable than others. The cytochromes were first observed at an early stage in the history of biochemistry [3], because of their sharp, intense visible absorption bands in the reduced state. Using a simple spectroscope, the three types of cytochromes, a, b and c, could readily be distinguished on the basis of their absorption maxima. By following the oxidation and reduction of cytochromes under different physiological conditions, much was learnt in the 1930's about their role in respiration in a wide range of organisms [4].

These and subsequent studies showed the importance of cytochromes in many of the enzyme systems of oxidation and reduction, and particularly the vital energy-producing processes of electron transfer: the respiratory chain of mitochondria, by which most foodstuffs are ultimately oxidized [5], and the photosynthetic electron transport chain of plants, by which light energy is used to split water and produce reducing equivalents for fixation of carbon dioxide [6]. In both cases the electron transfer is connected to the formation of ATP, the universal energy currency of the cell.

The flavoproteins, which also have characteristic visible absorption and fluorescence properties, were also detected in the 1930's as components of oxidation-reduction systems [7]. More recently, three other types of electron-transferring components, namely quinones, copper proteins and iron-sulphur proteins, have been detected in the same electron transfer systems of animals, plants and bacteria. These components have relatively inconspicuous optical absorption spectra and studies of them have been greatly assisted by the application of another spectroscopic technique, electron paramagnetic resonance (EPR) [8].

EPR spectroscopy is less sensitive than spectrophotometry, so that concentrated samples are needed. Moreover the measurements are normally made on frozen solutions at low temperatures (except for free radicals). In order to follow kinetic changes in the oxidation-reduction state of components, it is necessary to use rapid freezing [9], a technique which has so far been applied mainly to simpler enzyme systems. Nevertheless, the EPR method can be applied to observe many electron-transferring components. Particularly successful have been studies on the iron-sulphur centres, which give rise to signals around $g = 1.94$ in the reduced state. Spectra of different centres can be distinguished on the basis of their lineshape and temperature dependence, and by changing the redox potential of the sample [10, 11].

2. The iron-sulphur proteins. — In the last few years, iron-sulphur proteins have been shown to have a previously unsuspected importance in biochemical systems [12-14]. Table 1 lists typical examples of the large number of iron-sulphur proteins that have been isolated or detected. It is now believed that there are at least 12 different iron-sulphur centres in the mitochondrial respiratory chain (in addition to 6 types of cytochrome) [15]. At least four iron-sulphur centres have been detected in the chloroplast photosynthetic electron transport chain, where they are involved in the primary processes of capturing light energy [16, 17]. Nitrogenase, the enzyme that fixes atmospheric nitrogen to ammonia [18, 19] and hydrogenase [20, 21] which catalyses the reduction of water to hydrogen, are both iron-sulphur proteins. In addition, iron-sulphur proteins are involved in processes as diverse as microbial fermentation, and the formation of steroid hormones in adrenal glands. EPR spectroscopy has been of great value in detecting the iron-sulphur centres in these systems, and is now being used to determine how they work.

As with spectrophotometry, biochemists often use EPR spectroscopy as a fingerprint technique to identify different types of paramagnetic metal-containing species. However the EPR spectra also contain a considerable amount of structural information, even though they are usually powder spectra because the samples are amorphous. This structural information has been invaluable in determining the chemical nature of new species. For example it was the low $g$-values of the signal from reduced spinach ferredoxin that gave the clue to the spin-coupled arrangement of iron atoms in the iron-sulphur proteins [22].

Any spectroscopic technique is limited in the type of compound that it can observe. For example, the enzyme system in plants that splits water to oxygen, and thereby produces reducing equivalents for photosynthesis, contains manganese, but this cannot be
detected by EPR or optical absorption. Therefore we know relatively little about how this important system works. For all we know, there may be other components that we cannot see at present. This could only be resolved by separating and reconstituting the whole system from purified components. Meanwhile any new spectroscopic techniques that are applicable to biological systems are valuable.

The determination of the structure of the iron-sulphur proteins is a good example of the application of spectroscopic techniques. Initially the work was mostly done on the simplest iron-sulphur proteins, the ferredoxins and rubredoxins, since they can readily be obtained in the pure state in reasonable quantities. The results obtained from studying these proteins however, can be extended to iron-sulphur centres in complex biological systems.

Isolation of ferredoxins from plants was first reported in 1952 [23] and from bacteria in 1962 [24]. Both had similar redox potentials and biological activity, but to the biochemist's eye, the two types of protein were obviously different because the former type were red in colour while the latter were greenish-brown. In fact this difference in colour reflected a fundamental difference in structure. We now recognise that the plant ferredoxins contain [2 Fe-2 S] centres (i.e. two atoms of iron and two of acid labile sulphide while the bacterial ferredoxins contain [4 Fe-4 S] centres. In addition the rubredoxins, which are found in bacteria, contain a single iron in each centre, bound to non-labile cysteine sulphur atoms. Figure 1 shows the arrangements of iron and sulphur atoms that are believed to exist in the three types of proteins.

As often happens in protein chemistry, straightforward chemical analysis of the ferredoxins proved to be difficult, because of the large size of the molecules and their instability. In the case of the bacterial ferredoxins, of the clostridial variety, even the number of iron and sulphide atoms was a matter of dispute until in 1970 a value of eight iron and eight sulphide atoms was decided upon [25]. The usual chemical treatments, designed to remove the chromophores for chemical analysis, resulted in decomposition (though they have subsequently been extracted by the process of core extrusion [26]).

3. **Spectroscopy of the 2Fe-2S proteins.** — The determination of the structure of the two-iron ferredoxins by X-ray crystallography has not so far been possible because of the difficulty in obtaining large enough crystals. Fortunately these proteins provided a good subject for a wide range of spectroscopic methods, each giving information about certain features of the molecule.

The spectroscopic techniques provide us with numerical data. To interpret them, we need to set up a model of what the compound might be, and see whether its predicted properties agree with the spectroscopic values. In the case of the two-iron ferredoxins, the spectroscopic data did not agree with those of any known compound of iron, so a hypothetical model was needed. The model of Gibson et al. [22] fulfilled this requirement. They started from the observation that spinach ferredoxin has an EPR spectrum only in its reduced form [27, 28], and the suggestion of Brintzinger et al. [29] that the iron atoms are bridged by labile sulphide atoms. They proposed that in oxidized ferredoxin the iron atoms are both high-spin ferric, antiferromagnetically coupled via the sulphur ligands to give a diamagnetic ground state. In reduced ferredoxin, one iron atom is high-spin ferrous so that the ground state is \( S = \frac{1}{2} \). From this elegant hypothesis, Gibson et al. [22, 30] were immediately able to explain the EPR g-values, and to predict a number of properties of the ferredoxin, which have subsequently been confirmed, namely the non-Curie Law behaviour of the magnetic susceptibility of oxidized ferredoxin [31], the existence of d → d transitions in the infrared region [32], and even the fact that the mechanism of electron spin relaxation would be an Orbach [33] process [34].

The results from a wide range of physical measurements of the two-iron ferredoxins have been summarized by Palmer [35]. Nuclear hyperfine splitting in the EPR spectra of isotopically substituted ferredoxins showed that two iron and two labile sulphide atoms were involved in the active site with just one unpaired electron [36, 37]. More quantitative information about the hyperfine interactions in \(^{57}\)Fe-substituted ferredoxins, in the form of components of the A-tensor, could be obtained from electron-nuclear double resonance (ENDOR) spectroscopy [8, 38].

![FIG. 1. — Structures of iron-sulphur centres. From [85].](image-url)
$^{57}\text{Fe}$ Mössbauer spectroscopy proved to be a powerful probe of the iron atoms, particularly since it could be applied to the oxidized as well as the reduced state. From the chemical shift and quadrupole splittings, the proposed valence states of the iron atoms were confirmed. The antiferromagnetic coupling was demonstrated by the presence of both positive and negative hyperfine fields [39-41]. By using the values of the $A$-tensor, Dunham et al. [41] were able to computer-fit the Mössbauer spectra, and obtain further information about the spectrum of $\text{Fe}^{2+}$ in the reduced plant ferredoxins, which is highly anistropic. Since the ground state for $\text{Fe}^{2+}$ was $d_{x^2-y^2}$, they concluded that the $\text{Fe}^{2+}$ is in tetrahedral coordination, with axial and rhombic distortion.

4. Preparation of samples for spectroscopy. — Most of these spectroscopic techniques are not available in the average biochemistry laboratory and the interpretation of the results is within the realm of physics or chemistry. Therefore experiments of this type are often a collaboration between the biochemists who supply the sample and the spectroscopists who make the measurements and (hopefully) interpret the results. The measurement of $^{57}\text{Fe}$ Mössbauer spectra of a ferredoxin illustrates the type of problems that need to be overcome. For biochemists the main problems are concerned with the preparation of pure, concentrated samples free of contamination [42, 43]. For the spectroscopists they are high sensitivity of instrumentation needed, and the complexity of the resulting spectra, which may include contributions from several types of iron atoms.

The sensitivity of Mössbauer measurements depends on instrumental parameters such as counting geometry and strength of source, and on the broadness of the spectrum. For a reasonably sensitive system the minimum quantity of sample required is of the order of 1 µmole of each species of $^{57}\text{Fe}$ [43]. For a two-iron ferredoxin of molecular weight 11.000 (which is small for a protein) at natural enrichment of $^{57}\text{Fe}$ (2.2 %) this would represent 500 mg of protein. This represents the product from about 30 kg of spinach, for example, and in any case would be difficult to concentrate to a sample volume of 1 ml. However if the iron is replaced by 90 % enriched $^{57}\text{Fe}$, the quantity of protein required is only 12 mg. Enrichment therefore offers great advantages. In some favourable cases $^{57}\text{Fe}$ can be introduced by growing the organism on $^{57}\text{Fe}$ and extracting the proteins. This was done in the case of haemoglobin [43] for example, where rats were deprived of iron to deplete their resources of natural $^{57}\text{Fe}$, and then injected with ferric citrate containing 80 % enriched $^{57}\text{Fe}$. Red blood cells taken subsequently contained haemoglobin at about 50 % enrichment. This was a favourable case, because about two-thirds of the iron in a rat is in the form of haemoglobin. By contrast all of the electron-transfer proteins represent something like 2 % of the total iron and therefore injection of $^{57}\text{Fe}$ would result in a very inefficient conversion. Most of the other cases of natural enrichment have been in bacteria; they can readily be grown on a defined medium, and a high yield of the required protein can be obtained by selecting the right type of organisms and growth conditions.

An alternative procedure is to purify the protein first, then to remove the iron and replace it with $^{57}\text{Fe}$. The method in the case of the ferredoxins is surprisingly simple. The iron and labile sulphide are removed by treatment with acid, and the resulting apoprotein is reconstituted by incubation with a $^{57}\text{Fe}$ salt and sodium sulphide, in the presence of a thiol [45]. (Unfortunately this method has not so far been successfully applied to more complex iron-sulphur proteins). The protein is then purified by column chromatography.

The reconstitution procedure introduces dangers of contamination with excess $^{57}\text{Fe}$. Even if chemical analysis shows the right amounts of iron to be present in the sample, some of it may be in an altered conformation. Such contamination in early spectra resulted in erroneous conclusions. In subsequent experiments the reconstituted protein was checked by a number of physical methods, including EPR and circular dichroism, to make sure that the conformation was not altered, and its activity in a bioassay with chloroplasts was also compared with the native protein [39]. After it had passed these tests, the protein showed Mössbauer spectra essentially identical, to those of the native protein (but much more intense). Unfortunately the Mössbauer spectrum is sometimes the most sensitive method of detecting contaminant iron. When determining the spectrum of a new protein therefore, it is wise to make measurements of a number of samples prepared in different ways. If all of the features of the spectrum remain the same, this makes it less likely that there is contaminant iron.

A problem that concerns biochemists is whether measurements made on frozen, extremely concentrated solutions of proteins, are relevant to their properties in the cell, where the concentration is lower by several orders of magnitude. The assumption must be made that the structure of the protein is unchanged from that under physiological conditions. When samples are thawed out or redissolved after Mössbauer measurements they are usually found to be unchanged in biological activity and spectroscopic properties, which indicates at least that the gamma-ray beam has little effect on the protein. For haem proteins it was found that freeze-dried samples had undergone some distortion of the protein molecules, compared with frozen aqueous solutions [43]. This is perhaps not surprising since the structure of a protein is maintained principally by hydrophobic and hydrophilic interactions. Even with aqueous solutions, changes in protein structure may take place on freezing due to high local concentrations of salt or changes in pH. These considerations must always be borne in mind when applying a new spectroscopic technique.
5. Spectroscopy of the \([4 \text{Fe-4 S}]\) proteins. — The proteins, such as \(C.\ pasteurianum\) ferredoxin, that contain \([4 \text{Fe-4 S}]\) centres, presented more of a challenge to spectroscopic techniques such as Mössbauer spectroscopy, because of the greater number of different iron atoms in each molecule. Fortunately it was possible to determine the structure of two proteins of this type by X-ray crystallography.

The structure of the eight-iron ferredoxin from \(P.\ aerogenes\) was determined by Adman, Sieker, and Jensen [46]. The protein is a compact molecule, containing two cube-like clusters of four iron and four labile sulphur atoms, linked to cysteine sulphurs from the polypeptide chain (Fig. 2).

It is probable that other eight-iron ferredoxins have a similar structure. The centres of the clusters are just 1.2 nm apart. Each cluster accepts one electron on reduction, giving rise to an EPR signal centred at \(g = 1.96\).

The structure of the four-iron high-potential iron-sulphur protein (HiPIP) from the purple photosynthetic bacterium \(C.\ pasteurianum\) was determined by Carter and Kraut [47]. Although the protein conformation was totally different from \(P.\ aerogenes\) ferredoxin, HiPIP proved to contain an almost identical iron-sulphur cluster. This was not expected, since HiPIP has totally different redox properties. Its redox potential is much more positive, and it shows an EPR signal centred at \(g = 2.04\) in the oxidized rather than the reduced state.

The apparently paradoxical redox behaviour of the \([4 \text{Fe-4 S}]\) clusters in HiPIP and the ferredoxins, has been rationalized by the three-state hypothesis of Carter et al. [48]. In this, the centres in oxidized ferredoxin and reduced HiPIP are in an equivalent nonmagnetic oxidation state (called here \(C^2\)).

In ferredoxin the cluster can be reduced to the paramagnetic \(C^2\) state, while in HiPIP it can normally only be oxidized to the paramagnetic \(C\) state. In support of this scheme it has subsequently been found that the centre in ferredoxin can be super-oxidized to a \(C\) state by potassium ferricyanide [49] while HiPIP can be super-reduced to a \(C\) state if the protein is unfolded by treatment with dimethylsulphoxide (DMSO) [50], or by rapid heat treatment (R. Cammack and H. Rupp, unpublished).

A valuable clue to the state of the iron in these \([4 \text{Fe-4 S}]\) clusters was provided by the synthesis, by Holm and his group [51], of a series of analog compounds \([\text{Fe}_4\text{S}_4(\text{RS})_4]^2-\) which were found to have almost identical cube-like structure to those in \(P.\ aerogenes\) ferredoxin and \(C.\ pasteurianum\) HiPIP. As prepared, these compounds are in a diamagnetic state, corresponding to the \(C^2\) state in oxidized ferredoxin and reduced HiPIP. The ionic charge on the compounds indicated formal valences for the iron atoms of \(2\text{Fe}^{3+} + 2\text{Fe}^{2+}\). However, by a number of spectroscopic criteria, the iron atoms in these compounds were found to be indistinguishable, indicating a high degree of electron delocalization [52]. As will be seen later, the iron atoms in the iron-sulphur proteins also show evidence of delocalization, although the iron atoms are not exactly equivalent. Electrochemical experiments showed that these analog compounds could be reduced to the \(C^3\) state and oxidized to the \(C^2\) state [51]. Subsequently Holm and his group have also synthesized analogs of the \([2 \text{Fe-2 S}]\) centre in plant ferredoxin [53], and the sulphur-coordinated iron atom in rubredoxin [54].

Therefore with the \([4 \text{Fe-4 S}]\) proteins, we have a good chemical model with which to compare their properties. It turns out that these properties can be considerably influenced by the protein environment around the cluster.

Figures 2 to 11 illustrate various ways of looking at the eight-iron ferredoxin from \(C.\ pasteurianum\). The structure of this molecule is probably very similar to that of \(P.\ aerogenes\) ferredoxin (Fig. 2). The \(\text{EPR spectra}\) of the paramagnetic \(C^-\) or \(C^2\) states illustrate some of the effects of the protein environment on the \([4 \text{Fe-4 S}]\) cluster. For comparison figure 4a shows the reduced state of the analog compound

\[
[\text{Fe}_4\text{S}_4(\text{Ph})_4]^3-.
\]

The spectrum of the four-iron ferredoxin from \(B.\ stearothermophilus\) (Fig. 3a) has similar \(g\)-values, but a small degree of rhombic symmetry indicating some distortion of the cluster. Figure 3b shows the spectrum of the eight-iron ferredoxin from \(C.\ pasteurianum\); only partly reduced so that most of the paramagnetism arises from molecules with just one centre reduced. Once again this is similar to the analog compound. However when both of the centres in \(C.\ pasteurianum\) ferredoxin are reduced (Fig. 3c), the spectrum becomes
more complex, owing to spin-spin interaction between the iron-sulphur clusters [55]. It is not clear whether this interaction has any physiological significance; the energy involved ($J \approx 10 \text{ cm}^{-1}$) is too small to affect the redox potential. However it suggests that a pathway for electron transfer may exist between the two centres.

Figure 3d shows the spectrum of Chromatium high-potential iron-sulphur protein (HiPIP). As already noted, this spectrum is observed in the oxidized ($C^-$) form of the protein, and the $g$-values are greater than the free-electron value of 2.0023.

The constraints on the iron-sulphur centres imposed by the protein environment are relaxed if the protein is unfolded by denaturing agents such as DMSO [56]. Above a certain critical concentration, DMSO causes an unfolding of the protein [57] and the EPR spectra then resemble more closely the spectra of the analog compound. The Mössbauer spectra also more closely resemble each other [62]. Figure 4 illustrates this effect for C. pasteurianum ferredoxin and Chromatium HiPIP. The centres are not destroyed by this process, since the original spectra of the proteins are restored when the DMSO concentration is decreased [50, 57].

Care must be taken in these experiments to keep the samples cold, and to exclude oxygen, otherwise irreversible denaturation takes place.

6. The optical absorption spectrum (Fig. 5) consists of a series of broad overlapping charge-transfer bands, with a characteristic peak at 390 nm in the oxidized form. On reduction of the molecule the absorption decreases significantly, consistent with less ferric character of the iron atoms. Because there

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**Fig. 3.** — EPR spectra of proteins containing [4 Fe-4 S] centres; a) reduced four-iron ferredoxin from Bacillus stearothermophilus; b) eight-iron ferredoxin from Clostridium pasteurianum, partially reduced with dithionite; c) Clostridium pasteurianum ferredoxin, fully reduced; d) oxidized HiPIP from Chromatium. Spectra were recorded at 20 K, with microwave power 20 mW, frequency 9.2 GHz.

**Fig. 4.** — EPR spectra of a) the reduced analog compound, [Fe$_4$S$_4$(SPh)$_4$]$^{3-}$ in 80 % DMSO/20 % H$_2$O — a sample of this compound was kindly provided by Dr. M. Gunther, Dr. B. Ridge and G. Christou of the University of Exeter; b) reduced C. pasteurianum ferredoxin in 80 % DMSO/20 % H$_2$O; c) reduced Chromatium HiPIP in 80 % DMSO/20 % H$_2$O. Conditions of measurement were as for figure 3.

**Fig. 5.** — UV/visible spectra of C. pasteurianum ferredoxin. Solid line, oxidized; dotted line, reduced.
are no sharp absorption peaks, the spectrum is difficult to distinguish in the presence of other chromophores. Thus EPR spectroscopy is a better method of studying these compounds in complex biochemical systems such as mitochondria. Nevertheless, the optical spectra of the purified iron-sulphur proteins contain information about the iron-sulphur bonding, and this has been used in the formulation of a molecular orbital model for the [4 Fe-4 S] clusters [59]. There is a potential for extending these studies by measurements in the infrared region, and by magnetic circular dichroism, which gives further information about the orbital levels.

7. Circular dichroism shows that the optical absorption is also associated with a weak optical activity (Fig. 6). Since the iron-sulphur cluster is essentially symmetrical, the asymmetry must arise from interaction with the protein, either by distortions of the

![Graphical representation of circular dichroism spectra](image_url)

**Fig. 6.** — Circular dichroism spectra of *C. pasteurianum* ferredoxin. Solid line, oxidized; dotted line, reduced.

![Graphical representation of ENDOR spectra](image_url)

**Fig. 7.** — Electron-nuclear double resonance (ENDOR) spectra of reduced *C. pasteurianum* ferredoxin. The dotted lines are of native protein, and solid lines for $^{57}$Fe-enriched. Spectra a, c and e were recorded at the field positions shown in the EPR insets. Spectra b, d and f are the difference spectra. The temperature of measurement was 4.2 K and the microwave power 100 mW; for a and b, frequency was 9.32 GHz; for c, frequency 8.74 GHz. Reproduced from [60].
cluster or the proximity of asymmetric carbon atoms. This technique is therefore capable of giving information, albeit qualitative, about the interaction of the cluster with the protein.

8. Electron-nuclear double resonance (ENDOR) spectra of $^{57}$Fe-enriched C. pasteurianum ferredoxin (Fig. 7) show the effects of hyperfine interactions with the $^{57}$Fe nuclei and with protons in the neighbourhood of the cluster. The contribution from the protons can be determined separately from measurements on the native protein containing $^{56}$Fe (Fig. 7a, c and e, dotted lines). The contribution from $^{57}$Fe can then be determined by subtraction (Fig. 7b, d and f). The $x$, $y$ and $z$ components of the $A$-tensor can be obtained, at least in principle, from the ENDOR spectra obtained at different $g$-values. In this case the spectra are complicated by the spin-spin interaction between the clusters which makes $x$, $y$ and $z$ components more difficult to identify.

For C. pasteurianum ferredoxin [60] only one set of hyperfine constants was observed, with values

$$A_x = 33 \pm 1, \quad A_y = 29 \pm 5, \quad A_z = 25 \pm 2 \text{ MHz}$$

corresponding to 1.62, 1.42 and 1.22 mm/s respectively in Mössbauer spectroscopic terms. This is in contrast to the 2 Fe-2 S proteins [38] in which it was possible to detect separate coupling constants for the Fe$^{3+}$ and Fe$^{2+}$ atoms, those for Fe$^{2+}$ being highly anisotropic. Therefore the iron atoms in the reduced [4 Fe-4 S] clusters are nearly equivalent. The values of the $A$-tensor are of considerable assistance in computer fitting the Mössbauer spectra of reduced ferredoxin [41].

9. X-ray photoelectron spectroscopy (ESCA) is capable of distinguishing the Fe$^{3+}$ and Fe$^{2+}$ states of iron, although the difference in binding energy is only small. In oxidized C. pasteurianum ferredoxin (Fig. 8) the iron atoms all appeared to be in the same valence state, to within the limits of resolution [61] as was found with the analog compound

$$[\text{Fe}_4\text{S}_4(\text{SCH}_2\text{Ph})_4]^{2-} [52].$$

In the spectra of the 3s electrons of C. pasteurianum ferredoxin [61], a splitting was observed (Fig. 8a) due to core polarization splitting. In addition, shake-up satellites were observed on the 2p lines (Fig. 8b). Both of these observations indicate that the iron atoms are magnetic although the molecule as a whole has a low magnetic susceptibility. This observation is consistent with antiferromagnetic coupling.

10. Mössbauer spectroscopy, being a local probe of the iron atoms, is a particularly suitable technique for investigating the valence and spin states in the iron-sulphur cluster. Some of the results on the Mössbauer spectroscopy of the [4 Fe-4 S] proteins are summarized in another paper [62] and in the proceedings of the previous Mössbauer conference [63]. The chemical shift can be used as a measure of the valence state of the iron atoms. The shifts of iron in other iron-sulphur proteins can be used as a calibration, since the atoms are all coordinated in the same way, approximately tetrahedrally to four sulphur atoms. The average chemical shifts of [4 Fe-4 S] clusters in a number of iron-sulphur proteins are summarized in table II. The shifts can be interpreted in terms of formal valences such as 2 Fe$^{3+}$ + 2 Fe$^{2+}$ for the C$^{2-}$ state, but, as with the analog compounds, there is considerable delocalization of the electrons, so that in most cases the iron atoms in the cluster are difficult to distinguish by this technique. However, there is still evidence for some dissimilarity between the iron atoms, particularly in the reduced four-iron ferredoxin from Bacillus stearothermophilus, where two distinct doublets of equal intensity are seen at 77 K [65]. The shifts of the two doublets are quoted in table II.

At 4.2 K, the Mössbauer spectra of the reduced [4 Fe-4 S] proteins show broadening due to magnetic hyperfine interactions [63]. However in reduced C. pasteurianum ferredoxin [66] in zero applied magne-
Mössbauer chemical shifts of the iron-sulphur proteins

At 77 K relative to pure iron metal at room temperature (quoted in millimetres per second)

<table>
<thead>
<tr>
<th>Protein</th>
<th>Chemical shift</th>
<th>Average valences</th>
<th>State</th>
<th>Formal valences</th>
<th>Ref.</th>
</tr>
</thead>
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<tr>
<td>Fe$^{3+}$ in rubredoxin</td>
<td>0.25</td>
<td>—</td>
<td>—</td>
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<td>—</td>
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<tr>
<td>Fe$^{3+}$ in adrenal ferredoxin</td>
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<td>—</td>
<td>—</td>
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<td>Fe$^{3+}$ in spinach ferredoxin</td>
<td>0.26</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Oxidized Chromatium HiPIP</td>
<td>0.31</td>
<td>4 Fe$^{2.75+}$</td>
<td>C$^-$</td>
<td>3 Fe$^{3+}$, 1 Fe$^{2+}$</td>
<td>[91]</td>
</tr>
<tr>
<td>Reduced Chromatium HiPIP</td>
<td>0.42</td>
<td>4 Fe$^{2.2+}$</td>
<td>C$^2$-</td>
<td>2 Fe$^{3+}$, 2 Fe$^{2+}$</td>
<td>[93]</td>
</tr>
<tr>
<td>Oxidized B. stearothermophilus ferredoxin</td>
<td>0.42</td>
<td>4 Fe$^{2.2+}$</td>
<td></td>
<td></td>
<td>[65]</td>
</tr>
<tr>
<td>Oxidized C. pasteurianum ferredoxin</td>
<td>0.43</td>
<td>4 Fe$^{2.2+}$</td>
<td></td>
<td></td>
<td>[66]</td>
</tr>
<tr>
<td>Oxidized Chromatium ferredoxin</td>
<td>0.41</td>
<td>4 Fe$^{2.2+}$</td>
<td></td>
<td></td>
<td>[68]</td>
</tr>
<tr>
<td>Super-reduced Chromatium HiPIP</td>
<td>0.59</td>
<td>4 Fe$^{2.25+}$</td>
<td></td>
<td></td>
<td>[99]</td>
</tr>
<tr>
<td>Reduced B. stearothermophilus ferredoxin</td>
<td>0.50</td>
<td>2 Fe$^{2.5+}$, 2 Fe$^{3+}$</td>
<td>C$^3$-</td>
<td>1 Fe$^{3+}$, 3 Fe$^{2+}$</td>
<td>[65]</td>
</tr>
<tr>
<td>Reduced C. pasteurianum ferredoxin</td>
<td>0.57</td>
<td>4 Fe$^{2.25+}$</td>
<td></td>
<td></td>
<td>[66]</td>
</tr>
<tr>
<td>Reduced Chromatium ferredoxin</td>
<td>0.54</td>
<td>4 Fe$^{2.25+}$</td>
<td></td>
<td></td>
<td>[68]</td>
</tr>
<tr>
<td>Fe$^{2+}$ in rubredoxin</td>
<td>0.65</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Fe$^{2+}$ in spinach ferredoxin</td>
<td>0.60</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

It is a feature of the low temperature spectra of all of the [4 Fe-4 S] proteins in their paramagnetic states, that as the intensity of the applied field is increased, certain hyperfine lines move in towards the centre of the spectrum, but others move outwards (Fig. 9d and e). This can be taken as evidence for both positive and negative hyperfine fields and hence of antiferromagnetic coupling within the [4 Fe-4 S] clusters. A more detailed interpretation of the Mössbauer spectra of theferredoxins is presented in a recent review [68].

11. The proton nuclear magnetic resonance (NMR) spectra of C. pasteurianum ferredoxin showed, in addition to the usual protein envelope of proton resonance, a series of individual proton resonances shifted to low field (seen in the expanded region of Fig. 10a). These were tentatively assigned to methylene protons of cysteine residues adjacent to the sulphurs that bind the iron atoms to the protein [69]. They are shifted by the contact interactions with the magnetic iron atoms. The extent of the shift depends on, among other things, the magnetic moment of the iron atoms. On increasing the sample temperature some of the resonances shift to lower field (Fig. 10b), indicating an increased magnetic susceptibility. This non-Curie law behaviour is expected for an antiferromagnetically coupled system [30]. Integration of the contact-shifted resonances indicates sixteen protons, in good agreement with the sixteen β-CH$_2$ protons attached to the two [4 Fe-4 S] clusters.

It is of interest that the resonances are well separated from each other in the spectrum of figure 10a. This is probably because the contact interaction is sensitive to the bond angles [69]. By contrast the analog compound [Fe$_4$S$_4$(SCH$_2$Ph)$_4$]$^{2-}$ shows a single contact-shifted peak [51]. The position of the contact-shifted resonances therefore is a sensitive monitor of subtle changes in the protein conformation around the iron-
sulphur cluster. For example it can be used to observe the unfolding of the protein around the cluster in DMSO solutions [57].

2. $^{13}$C NMR has also been used to investigate the amino acids around the iron-sulphur cluster in C. pasteurianum ferredoxin [70]. The method is inherently less sensitive than proton NMR, so that very concentrated samples are needed, and proton decoupling techniques are used to enhance the spectra. C. pasteurianum ferredoxin contains two aromatic amino acids, phenylalanine and tyrosine, each with its ring close to an iron-sulphur cluster. The $^{13}$C resonances of these aromatic rings are well separated from the rest of the NMR spectrum, being shifted by the paramagnetism of the iron-sulphur clusters. It is possible to assign the resonances to particular carbon atoms in the two aromatic rings (Fig. 11). The positions of the resonances change on reduction of the neighbouring $[4$ Fe-$4$ S$]$ cluster. This provides a way of following the oxidation and reduction of each individual cluster (other techniques such as EPR cannot distinguish between them). By following the positions of the aromatic resonances as the molecule was progressively reduced, Packer et al. [70], were able to show that the midpoint reduction potentials of the two clusters differ by about 10 mV.

13. Conclusions from the spectroscopic results. — We therefore have information from a large number of different spectroscopic techniques about the ferredoxin from C. pasteurianum and related proteins. From this a picture is beginning to emerge about the chemistry of the various oxidation states of the $[4$ Fe-$4$ S$]$ cluster. It is a mixed valence compound of iron, but is not a spin-trapped system like the reduced $[2$ Fe-$2$ S$]$ cluster.

Thomson [59] has proposed a molecular orbital model of the $[4$ Fe-$4$ S$]$ clusters, assuming a tetrahe-
dral arrangement of iron atoms. This model can explain the optical absorption spectra of the proteins, and the EPR g-values of reduced ferredoxin and oxidized HiPIP. A similar approach was used by Eicher et al. [71] to fit the Mössbauer spectra of *C. pasteurianum* ferredoxin. In practice, however, the cluster in these proteins is not perfectly symmetrical, since Mössbauer and NMR spectroscopy show that the iron atoms are inequivalent, and even in the analog compounds, there is evidence for distortion from tetrahedral symmetry so that the point group is more nearly tetragonal, D_{2d} [51, 52]. It is not clear whether the simple molecular orbital treatment holds under these conditions.

The model proposed by Dickson et al. [63] for the [4 Fe-4 S] cluster represents a different approach. The iron atoms are proposed to be antiferromagnetically coupled in pairs, with electrons hopping rapidly between the pairs of atoms (which in the extreme case is equivalent to delocalization). This model is successful in explaining many of the spectroscopic results, although it leaves some questions unanswered, such as why oxidized HiPIP has g-values greater than 2.00. A similar proposal has been made by Antanaitis and Moss [73] to explain the EPR spectrum of oxidized HiPIP, which appears to contain two components.

So far these techniques have mostly been applied to simple proteins. We have much to learn about the more complex iron-sulphur proteins of great biological importance, such as those listed in table 1. Although these proteins probably contain iron-sulphur clusters similar to those in the ferredoxins and rubredoxins (Fig. 1), there are appreciable differences in their properties, which are produced by the different protein environments around the clusters.

With regard to the iron-sulphur proteins in general, there are a number of problems to which spectroscopy may provide the answer:


As already noted, this information is difficult to obtain by direct chemical analysis, even when purified, intact protein is available. It would be better to have a spectroscopic method to distinguish the types of centre, particularly in systems that are labile or difficult to isolate. The optical absorption and circular dichroism spectra are usually characteristic of the type of centre, but may be masked by other chromophores in the system. As already noted, EPR spectra of the [4 Fe-4 S] ferredoxins can be very variable and are not diagnostic for this type of centre (Fig. 3), but the EPR spectra after reduction in 80 % DMSO solution [56], all resemble those of the analog compounds [74] (Fig. 4). The [2 Fe-2 S] proteins give another type of spectrum with a different temperature dependence [56] and therefore the spectra of samples reduced in DMSO solution can be used as a diagnostic criterion. This procedure has been used to show that the membrane-bound iron-sulphur proteins in Photosystem I of plant photosynthesis are of the [4 Fe-4 S] type [75]. This type of cluster is therefore not confined to bacterial systems. In this case, the presence of large quantities of chlorophyll would make spectrophotometric measurements impracticable.

An alternative approach is the core extrusion technique. The protein is treated with 80 % DMSO and the iron-sulphur centre is extracted by exchanging with suitable ligands. The nature and concentration of the resulting iron-sulphur complex are determined by spectrophotometry [26]. This technique has been applied to a number of ferredoxins and hydrogenase [21]. By the use of controlled conditions, both the EPR and spectrophotometric methods can give quantitative results.

13.2 How does the protein control the redox potential of the iron-sulphur centre? — Table I shows that the potentials of the iron-sulphur proteins can vary between +350 mV for *Chromatium* HiPIP to -594 mV for one of the [4 Fe-4 S] centres in Photosystem I. Part of the explanation lies in the fact that HiPIP undergoes oxidation-reduction between the C^- and C^2^- states, while the ferredoxins, and centres in Photosystem I undergo oxidation-reduction between the C^- and C^3^- states. The question then arises, how does the protein in HiPIP prevent the centre from reduction to the C^3^- state? From X-ray crystallographic evidence, the reduction of the [4 Fe-4 S] centre causes an expansion of the cluster dimensions. Carter et al. [72] proposed that the HiPIP molecule, which is firmly held together with hydrogen bonds, might physically prevent the centre from being reduced to the C^3^- state.

Kassner and Yang [76] proposed, on the basis of electrostatic considerations, that the ease with which an iron-sulphur centre is reduced will depend on its formal charge. This charge is calculated by adding up the charges on the iron and its ligands, and is equal to the charge on the equivalent analog compound. The ferredoxins, with formal charge -2 in the oxidized cluster, are therefore more difficult to reduce than rubredoxin with formal charge -1. However this model would predict similar midpoint potentials for rubredoxin (actually -60 mV) and HiPIP (+350 mV). Clearly other factors must be taken into account, such as delocalization of electrons onto the ligands, and the effective local dielectric constant in the protein around the cluster. The latter would clearly depend on the type and arrangement of amino acids around the cluster.

Other explanations may have to be invoked to explain the wide variation in potential of the ferredoxin-type iron-sulphur centres (i.e. those giving an EPR signal in the reduced state), which can have potentials as high as +280 mV for Rieske’s iron-sulphur protein in mitochondria [77, 78]. Moreover some proteins seem capable of altering the midpoint potential of the iron-sulphur centres as a part of their
The oxidation states that the centres can take up are described in terms of formal charge. This is calculated by adding the charge on the iron atoms (+2 or +3) to that of the ligands (−2 for sulphide, −1 for thiolate). Thus \([\text{4 Fe-4 S}]^{2-}\) corresponds to the diamagnetic \(C_2^-\) state of the 4 Fe-4 S cluster. FAD = flavin adenine dinucleotide; FMN = flavin mononucleotide.

<table>
<thead>
<tr>
<th>Protein</th>
<th>Source</th>
<th>Function</th>
<th>Molecular Weight</th>
<th>Type of Fe-S center</th>
<th>Midpoint Reduction Potential mV</th>
<th>Other Groups</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-Iron ferredoxin</td>
<td>Plants (e. g. Spinach), algae</td>
<td>Photosynthetic electron transfer</td>
<td>10.500</td>
<td>([\text{2 Fe-2 S}]^{2-;3-})</td>
<td>−420</td>
<td>—</td>
<td>[23, 35]</td>
</tr>
<tr>
<td>2-Iron ferredoxin (Adrenodoxin)</td>
<td>Adrenal glands (e.g. pig)</td>
<td>Hydroxylation of steroids</td>
<td>12.500</td>
<td>([\text{2 Fe-2 S}]^{2-;3-})</td>
<td>−270</td>
<td>—</td>
<td>[86, 35]</td>
</tr>
<tr>
<td>2-Iron ferredoxin (Putidaredoxin)</td>
<td><em>Pseudomonas putida</em> (aerobic bacterium)</td>
<td>Hydroxylation of camphor</td>
<td>12.500</td>
<td>([\text{2 Fe-2 S}]^{2-;3-})</td>
<td>−240</td>
<td>—</td>
<td>[87]</td>
</tr>
<tr>
<td>4-Iron ferredoxin</td>
<td>Bacillus (e. g. <em>B. stearothermophilis</em>)</td>
<td>?</td>
<td>8.500</td>
<td>([\text{4 Fe-4 S}]^{2-;3-})</td>
<td>−280</td>
<td>—</td>
<td>[65]</td>
</tr>
<tr>
<td>8-Iron ferredoxin</td>
<td>Anaerobic bacteria (e.g. <em>C. pasteurianum</em>)</td>
<td>Fermentation reactions</td>
<td>6.000</td>
<td>([\text{2 Fe-4 S}]^{2-;3-})</td>
<td>−390</td>
<td>—</td>
<td>[24]</td>
</tr>
<tr>
<td>Rubredoxin</td>
<td>Bacteria (e. g. <em>C. pasteurianum</em>)</td>
<td>Photosynthesis</td>
<td>9.000</td>
<td>([\text{2 Fe-4 S}]^{2-;3-})</td>
<td>−490</td>
<td>—</td>
<td>[88, 89]</td>
</tr>
<tr>
<td>HiPIP (High-potential) iron-sulphur protein</td>
<td><em>Chromatium</em></td>
<td>Photosynthesis</td>
<td>10.000</td>
<td>([\text{4 Fe-4 S}]^{1-;2-})</td>
<td>+350</td>
<td>—</td>
<td>[92, 93]</td>
</tr>
<tr>
<td>Hydrogenase</td>
<td>Bacteria (e. g. <em>C. pasteurianum</em>)</td>
<td>Formation or assimilation of (H_2)</td>
<td>60.000</td>
<td>([\text{4 Fe-4 S}]^{1-;2-;3-})</td>
<td>?</td>
<td>—</td>
<td>[20, 21]</td>
</tr>
<tr>
<td>Nitrogenase : Mo-Fe protein</td>
<td>Nitrogen-fixing bacteria blue-green algae</td>
<td>Fixation of (N_2) to (NH_3)</td>
<td>220.000</td>
<td>([\text{4 Fe-4 S}]^{?})</td>
<td>−30 Mo</td>
<td>—</td>
<td>[18, 19, 79]</td>
</tr>
<tr>
<td>Fe-protein</td>
<td>—</td>
<td>—</td>
<td>66.000</td>
<td>([\text{4 Fe-4 S}]^{2-;3-})</td>
<td>−290</td>
<td>—</td>
<td>[18, 19, 79]</td>
</tr>
<tr>
<td>Xanthine oxidase</td>
<td>Milk</td>
<td>Oxidation of xanthine</td>
<td>270.000</td>
<td>([\text{2 Fe-2 S}]^{2-;3-})</td>
<td>−343, −303</td>
<td>FAD, Mo</td>
<td>[94]</td>
</tr>
<tr>
<td><em>Mitochondrial Respiratory chain</em></td>
<td>Animals &amp; Plants</td>
<td>Oxidative phosphorylation</td>
<td></td>
<td></td>
<td>Approx. 7-iron sulphur centres, type unknown</td>
<td>—</td>
<td>[10, 15]</td>
</tr>
<tr>
<td>NADH-ubiquinone reductase</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>FMN</td>
<td></td>
</tr>
<tr>
<td>Succinate dehydrogenase</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>FAD</td>
<td>[95, 96]</td>
</tr>
<tr>
<td>Ubiquinone-cytochrome c reductase</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>cytochromes b &amp; c</td>
<td>[78, 97]</td>
</tr>
<tr>
<td>Primary acceptor complex of Photosystem I</td>
<td>Plants, algae</td>
<td>Photosynthesis</td>
<td></td>
<td></td>
<td></td>
<td>chlorophyll P 700 &amp; Primary acceptor « X »</td>
<td></td>
</tr>
</tbody>
</table>
biochemical function. The Fe-protein of nitrogenase, for example, changes its potential from $-290 \text{ mV}$ to $-400 \text{ mV}$ on addition of ATP [79]. The change is accompanied by a change in the EPR spectrum [80].

A relevant structural feature is the existence of NH-S hydrogen bonds which may help to stabilize the more negatively charged iron-sulphur clusters [72]. There are 3-5 of these bonds in HiPIP, 6 in rubredoxin and 15-18 in P. aerogenes ferredoxins [81]. The difference between ferredoxin and HiPIP may help to explain the difference in properties of their [4 Fe-4 S] clusters.

13.3 How does the protein control the reactivity of the iron-sulphur centre? — X-ray crystallography shows that the protein is wrapped around the iron-sulphur centres in such a way as to isolate them from the aqueous environment. This serves to protect the iron-sulphur cluster from decomposition by hydrolysis or reaction with oxygen. It also means that the protein can control the access of oxidizing and reducing agents to the centre. In some cases the reactivity of the iron-sulphur centre towards substrates is actually enhanced. For example, hydrogenase, the enzyme that catalyzes the reduction of water protons to hydrogen gas, consists simply of a protein and one or more [4 Fe-4 S] clusters (the number is not yet decided [20, 21]). Other [4 Fe-4 S] proteins that have a sufficiently low redox potential to carry out this reaction, do not produce hydrogen to an appreciable extent. What are the properties of the cluster in hydrogenase that enable it to catalyze the reaction?

The modification of the redox properties and reactivity of the iron-sulphur centre by the protein may be an example of $\text{entasis}$, a name used by Vallee and Williams [82] to describe the effects of structural constraints on the active site, such as a metal-binding centre in a protein, which enhance its reactivity. Examples of distortion caused by these constraints can be seen in the spectroscopic properties of the iron-sulphur proteins [56]. Those spectroscopic methods, such as EPR, NMR and circular dichroism, which are sensitive to subtle changes in the cluster and its ligands, may help to determine the nature of the modifications.

14. Further applications of Mössbauer spectroscopy. — The investigations of the nitrogenase proteins of Klebsiella pneumoniae by Smith and Lang [83] is a bold attempt at elucidating the arrangements of the iron-sulphur centres, and the electron transfer reactions that take place in this complex system. It is a good example of collaborative work between the biochemist and physicist. The unravelling of the spectra, which contain contributions from at least three types of iron species, was achieved by measurements over a wide range of temperature and magnetic field, on samples prepared in different oxidation states and in the presence of substrates of the enzyme. One of the species present in the Mo-Fe protein appeared to be similar to the [4 Fe-4 S] centre in HiPIP. This particular centre is reduced under conditions where nitrogen fixation takes place, i.e. the presence of the Fe-protein, ATP and a reducing agent.

Mössbauer spectroscopy is not easily applied to the study of biochemical reactions, since the samples must be solid, and measurements are slow. Possibly, use will be made in the future of the rapid-freezing method for trapping the intermediate stages of the reaction [9].

One type of reaction that can be studied in the solid state is photochemical processes. A recent study of low temperature photodissociation of complexes of haemoglobin and myoglobin [84] is an example. The primary processes of plant and bacterial photosynthesis provide potential subjects for future study. Illumination of samples of Photosystem I particles from plant chloroplasts, at temperatures as low as 4.2 K, results in the photo-oxidation of the chlorophyll pigment P 700 and reduction of the membrane-bound iron-sulphur centres. These processes can be followed by EPR [6]. Also in the Photosystem I complex there appears to be another component, referred to in table I as $\text{X}$, that can accept electrons reversibly from P 700 if the other iron-sulphur proteins are already chemically reduced. This component, which has so far only been detected by its EPR spectrum [6, 100] appears to be the best candidate for the elusive primary electron acceptor of plant photosynthesis. The elucidation of the chemical nature of $\text{X}$, which is involved in the primary energy-producing reaction on which all life depends, seems a worthwhile target for future spectroscopic investigations.

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References


