RESONANCE RAMAN SCATTERING ON THE HAEM GROUP OF CYTOCHROME c

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SUMMARY

Resonance Raman spectra of the haem group of 8 x 10^{-5} M horse heart ferro- and ferricytochrome c solutions have been obtained. The spectra are almost identical to that of haemoglobin. The frequency of the Raman line near 1370 cm^{-1}, which in haemoglobin is sensitive to the position of the haem iron, indicates that the iron atom of cytochrome c lies in the plane of the porphyrin for both oxidation states.

Introduction

Here we report resonance Raman scattering from vibrational modes of the haem group of ferro- and ferricytochrome c in aqueous solution. Resonance Raman scattering (1,2) occurs when the exciting light falls within an electronic absorption band of the investigated molecule. The intensity of the resonantly scattered light is several orders of magnitude higher than in normal Raman scattering and makes it feasible to obtain Raman spectra of biomolecules in aqueous solution at biologically relevant concentrations of about 10^{-5} M. Like in haemoglobin (3,4) only the haem group of cytochrome c absorbs in the visible and near ultraviolet region. Thus it is possible to investigate by means of resonance Raman scattering exclusively the vibrations of the chromophore of cytochrome c without interference by scattering of the huge surrounding protein.

Recently we had obtained (3,4) resonance Raman spectra of haemoglobin and some of its derivatives. Cytochrome c, one member in the respiratory electron transport chain, is

another haemprotein containing the same prosthetic group, iron protoporphyrin IX (haem). This group, however, is attached to the protein not only by one axial ligand (ε-imidazole nitrogen of histidine) of the iron atom as in haemoglobin. There exists, in both oxidation states (6,7) a second axial ligand, the sulfur atom of methionine 80 which is part of the protein. Additionally, the haem is linked to cysteine 14 and cysteine 17 by two thioether bonds, thereby destroying the two vinyl double bonds at the periphery of the protoporphyrin IX.

Experimental

Horse heart cytochrome c was purchased from Boehringer Mannheim and used without further purification. It was oxidized with ferricyanide and reduced with dithionite. Complete oxygenation and reduction, respectively, was tested by the absorption spectrum. The 4880 Å line of an argon- ion-laser with average power of 500 mW was used for excitation. The scattered light was analysed by a double monochromator and detected by a EMI 9558 photo tube followed by a lock-in amplifier. During all measurements the cytochrome c solutions were kept at a temperature of 4 °C.

Results and Discussion

The Figure shows the resonance Raman spectrum of ferri-cytochrome c in the range from 300 to 1700 cm^{-1}. It is almost identical to that of haemoglobin (3,4). Because of the similarity of both the spectra and the scattering entities, the haem group, of cytochrome c and haemoglobin it seems reasonable to take over the tentative assignments for haemoglobin (4) to the spectra of cytochrome c: The two lines below 500 cm^{-1} should be due to Fe-N-stretching vibrations or

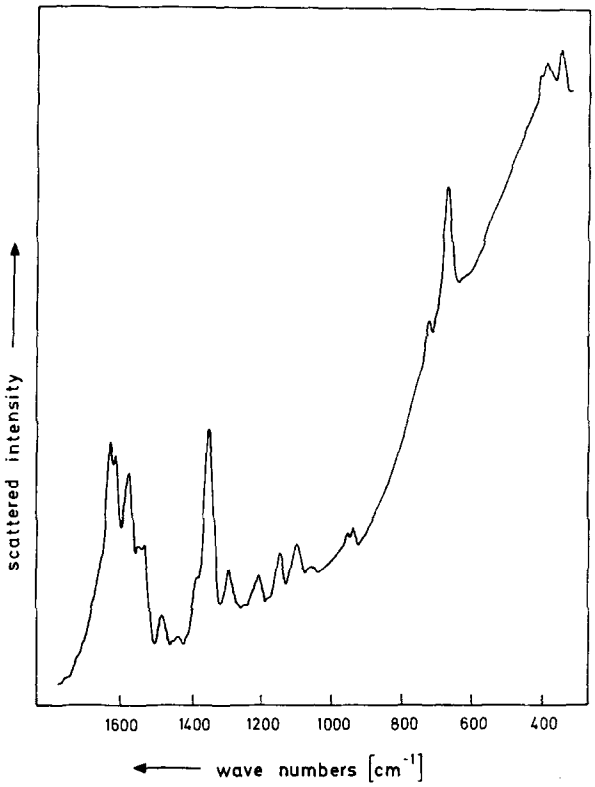

Figure. Resonance Raman spectrum of 8 x 10^{-5} M ferricytochrome c solution, pH 6.5, at 4 °C. Resolution 6.5 cm^{-1}. Abscissa: Raman frequency shift from exciting line in wave numbers (cm^{-1}); ordinate: intensity of resonantly scattered light in arbitrary units.

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at least to low lying porphyrin skeletal vibrations coupled with an iron-nitrogen stretching mode. The lines at 687 cm^{-1} and 744 cm^{-1} are attributed to out-of-plane deformation modes of the porphyrin. The line at 744 cm^{-1} is remarkably weaker than the corresponding lines in both ferrous and ferric haemoglobin. The line at 1230 cm^{-1} is believed to arise from an in-plane bending vibration of the methine hydrogens. The strongest line of the spectrum, discussed in detail below, appears at 1372 cm^{-1} and is assigned to a =C-N-stretching vibration. The corresponding line in haemoglobin (at 1376 cm^{-1} in oxyhaemoglobin) shows (3) a striking frequency shift upon

Table. The observed vibrational frequencies in wave numbers (cm^{-1}) of the haem group of horse heart ferri- and ferrocytochrome c solutions. Figures in parentheses are relative intensities of the Raman lines, normalized to a value of 10 for the strongest line in each spectrum. The symbol sh denotes a shoulder. ν means stretching, δ bending and π out-of-plane deformation vibrations.

Ferricytochrome c	Ferrocytochrome c	Assignment
352 (2)	352 (2)	
404 (2)	392 (2)	ν (Fe-N)
	441 (0)	
	638 (1)	
687 (5)	691 (6)	π(Ring)
744 (1)	749 (1)	π(Ring)
	794 (0)	
971 (1)	971 (1)	
986 (0)	988 (0)	
1083 (0)	1086 (0)	
1124 (2)	1126 (2)	
1173 (2)	1175 (2)	
1230 (2)	1230 (1)	δ(C-H)
1243 (0sh)		
1312 (2)	1314 (1)	
1372 (10)	1365 (10)	ν(=C-N)
1401 (1)	1401 (2)	
1458 (0)		
1500 (2)	1494 (2)	
1543 (1)	1548 (6)	
1560 (1)	--	ν(C=C) &
1586 (5)	1586 (6)	ν(C=N)
1621 (2)	1621 (7)	
1634 (6)	--	

deoxygenation. This led us to the formulation (4) of a structure-frequency-correlation monitored by this line. The lines between 1500 cm^{-1} and 1640 cm^{-1} are attributed to the presumable heavily mixed C=C- and C=N-stretching vibration of the porphyrin.

All observed frequencies are listed in the Table.

The observed unusually high depolarisation ratios of some Raman lines of both ferro- and ferricytochrome c and their dependence upon the wavelength of the exciting laser line will be published elsewhere. They do not agree with those reported recently by Strekas and Spiro (5).

Dickerson et al. have revealed the threedimensional structure of both oxidized (6) and reduced (7) horse heart cytochrome c by x-ray analysis. But nothing has been said about the precise position of the iron atom relative to the plane of the haem or its probable movement when its oxidation state is altered. In the case of haemoglobin the movement of the iron atom is known to be important for its ligand binding properties (8). The haem iron of cytochrome c is low spin in both oxidation states. Carrying over the well established arguments developed by Hoard (9,10) concerning the radius of the iron and that of the "central hole" in the porphyrin, the iron is expected to lie in the plane of the four porphyrin nitrogens. This position should be held in both oxidation states for the octahedral covalent bond radii of Fe^{2+} and Fe^{3+} differ by only 0.02 Å. Furthermore there exists evidence from x-ray analysis (7) that the amino-acids methionine 80 and histidine 18 which supply the two axial ligands to the iron, do not move in going from ferri- to ferrocytochrome c. This seems to imply that the iron, too, does not change position.

We now apply to cytochrome c the above mentioned structure-frequency-correlation derived (4) for ferrous haemoglobin and which holds as well for ferric haemoglobin and chlorohaemin (4). It is based on the observation that the

frequency of the Raman line near 1370 cm^{-1}, assigned to a =C-N-stretching vibration, seems to be sensitive to the position of the iron atom relative to the porphyrin plane. However, care must be taken in comparing different haemoproteins. Therefore we investigated the model compound bis-pyridin-haemin (BPH), which, conferring to the stereochemical environment of the iron atom seems to us as closely related to cytochrome c as is chlorohaemin to aquomethaemoglobin. In BPH the iron is as well low spin and axially twofold ligated as in cytochrome c. Moreover, the iron of BPH is believed to be in-plane in both oxidation states like the low spin iron of the very similar complexes bis(imidazole)-α,β,γ,δ-tetraphenylporphinatoiron (III) chloride (11) and bis(piperidine)-α,β,γ,δ-tetraphenylporphinatoiron (II) (12). For the compared molecules cytochrome c and BPH the frequency values of the line under consideration are

oxidation state of Fe	Cytochrome c	BPH
2+	1365 cm^{-1}	1363 cm^{-1}
3+	1372 cm^{-1}	1372 cm^{-1}

The very good agreement of corresponding frequencies of cytochrome c and the reference molecule BPH is interpreted as experimental evidence that the iron atom of cytochrome c lies in the plane of the porphyrin for both oxidation states and therefore do not move when its oxidation state is altered.

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