A RESONANCE RAMAN STUDY ON THE NATURE OF CHARGE-TRANSFER INTERACTIONS IN BUTYRYL CoA DEHYDROGENASE

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1. Introduction

Butyryl-CoA dehydrogenase (BCD) (EC 1.3.99.2) is a flavoprotein that catalyses the first step in fatty acid β-oxidation. When isolated from various sources, the pure enzyme has a characteristic green colour [1–3], due to a long-wavelength absorption band centred at 710 nm. We have proposed that a new chemical species, CoA persulphide, tightly-bound at the enzyme's active site, is the donor in a charge-transfer interaction with the FAD prosthetic group [4,5]. Complexes with long-wavelength absorption are also formed between the enzyme and various acyl-CoA compounds, including acetoacetyl-CoA, which gives a grey–green complex with an absorbance maximum at 580 nm [6].

Resonance Raman (RR) spectroscopy can reveal the vibrational spectra of the chromophores of large biomolecules, and has been acknowledged as a powerful tool for studying the structural details of these chromophores [7]. RR studies of charge-transfer complexes of 'old yellow enzyme' with phenol derivatives [8,9], and of D-amino acid oxidase with benzoate derivatives [10], have shown the usefulness of the technique for elucidating interactions between enzyme-bound flavin and other ligands. The RR study on the reaction intermediates of D-amino acid oxidase [11] also shows the value of the technique in the determination of the molecular structure of a ligand.

Here, we have applied the technique to the 'green' and 'grey–green' forms of BCD by exciting Raman scattering in the charge-transfer band of each complex. We report new information on the nature of the charge-transfer interaction in these 2 complexes of BCD.

2. Materials and methods

Butyryl-CoA dehydrogenase was purified from *Megasphaera elsdenii* as in [12]. Coenzyme A was purchased from Sigma. Other reagents were from BDH.

Yellow enzyme (fig.1) was prepared from the native green form by prolonged anaerobic dialysis followed by air re-oxidation [6]. The grey–green complex (fig.1) was made by adding to the yellow enzyme an amount of acetoacetyl-CoA equimolar with the bound FAD. To give full formation of the green complex (fig.1), native enzyme was saturated with CoA persulphide by incubation with equimolar CoASH and a 15-fold excess of Na_2S [5]. Excess ligand was removed by gel filtration on Sephadex G-25 followed by ammonium sulphate precipitation. All samples were stored as precipitates in 75% ammonium sulphate. Before use, each sample was dialysed to give a final concentration of 1.15% ammonium sulphate in 0.1 M sodium phosphate buffer (pH 7.0).

RR spectra were obtained with a JASCO R-800 Raman spectrometer (Japan Spectroscopic Co.) and a He-Ne laser (Kinmon Electric, model KLG-103). The wave number scale of the spectrometer was calibrated with the Raman lines of indene [13]. The laser power used was ~40 mW at sample point. Spectral resolution was 2 cm^{-1} at 632 nm.

3. Results and discussion

Fig.2 shows the Raman spectra of BCD, its green complex and the grey–green BCD-acetoacetyl-CoA complex excited at 632.8 nm. All of the samples contained 1.15% (w/w) of $(NH_4)_2SO_4$ and the

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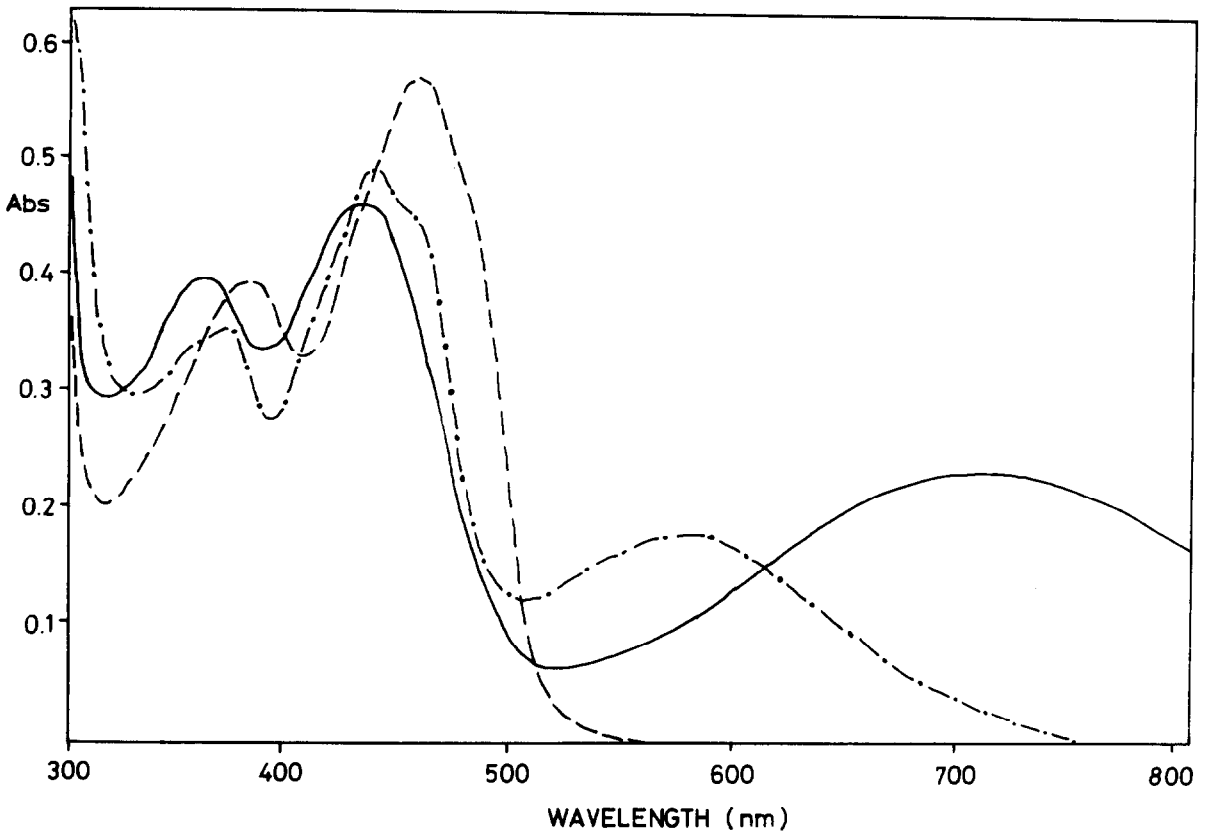

Fig.1. Spectral forms of butyryl-CoA dehydrogenase. Yellow BCD (– – –) has no long wavelength absorption. The BCD-acetoacetyl-CoA complex (–.–.) and native BCD (——) both show long wavelength absorption, with bands centred at 580 nm and 710 nm, respectively. The absorbance of all enzyme samples (44 μM) was measured using a 1 cm lightpath.

981 cm^{-1} line of SO_4^{2-} thus served as an internal standard for the intensity of Raman lines. Raman lines derived from the free ligands are not expected under these conditions.

Yellow BCD has no absorption around 632.8 nm (fig.1), and no Raman line of isoalloxazine was observed upon excitation at 632.8 nm (fig.2). On the other hand, the complexes have broad absorption bands around 632.8 nm, characteristic of charge transfer, and their Raman spectra excited at 632.8 nm included the Raman lines of both FAD and ligands.

In the RR spectrum of the green complex (fig.2) the Raman lines at 1585 and 1550 cm^{-1}, derived from the isoalloxazine ring, were extensively enhanced. The enhanced lines at 503, 447 and 396 cm^{-1} were considered to be derived from the ligand. The most enhanced line at 503 cm^{-1} is probably derived from the S–S stretching mode [14,15]. This result indicates that the charge-transfer band results from direct interaction between flavin and the S–S region of the ligand. This supports the view that the charge-transfer donor in the green form of BCD is a CoA persulphide species [4,5].

The lines at 1585 and 1550 cm^{-1} have been shown to involve the vibrational displacements of N(5) and C(4a) atoms of isoalloxazine. Therefore the S–S region of CoA persulphide as an electron donor may lie along the N(5)–C(4a) bond of isoalloxazine as an electron acceptor.

The Raman spectrum of a complex of pig liver general acyl-CoA dehydrogenase and acetoacetyl-CoA has been measured [16]. It was proposed that acetoacetyl-CoA interacts with the dehydrogenase flavin in the C(4a)–N(5) region and that this interaction might involve the C_2-methylene of the acyl-CoA derivatives as well as the carbonyl of the thiol ester [17].

In the RR spectrum of the BCD–acetoacetyl-CoA complex with excitation at 632.8 nm, which is in the region of the charge-transfer band (fig.2C), the resonance enhancement of lines at 1584,1553,1352 and 610 cm^{-1} derived from isoalloxazine was observed. The resonance enhancement for the lines around 1350 and 610 cm^{-1} suggests that the charge-transfer interaction in this complex is associated with the C(4a)–C(10a)–N(1)–C(2) region in addition to the C(4a)–N(5) region implicated in the case of the green form, since the Raman line around 1350 cm^{-1} has been assigned to C(4a)–C(10a)–N(1)–C(2) stretching and the line around 610 cm^{-1} involves the vibrational displacement of the C(2) atom of isoalloxazine

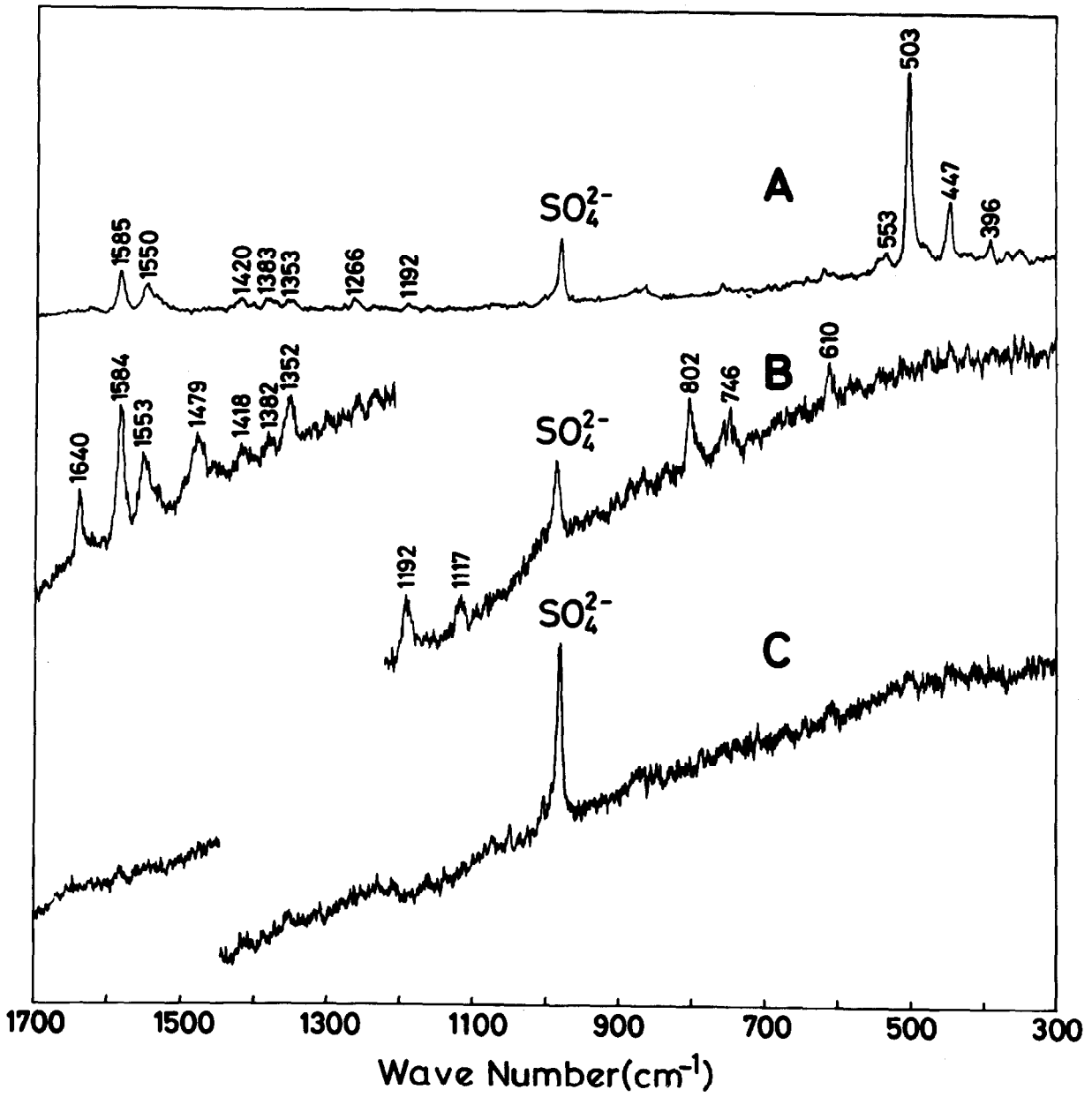

Fig.2. Resonance Raman spectra of butyryl-CoA dehydrogenase excited at 632.8 nm: (A) green form, 5.1 × 10^{-4} M; (B) BCD–acetoacetyl-CoA complex, 5.3 × 10^{-4} M; (C) yellow form, 5.8 × 10^{-4} M.

[18]. The lines at 1640,1479,802 and 746 cm^{-1} are considered to be derived from the enol form of acetoacetyl-CoA. The line at 1640 cm^{-1} is probably due to C=O or C=C stretching, but this cannot be definitely determined from these data. To assign these lines, studies with isotopically substituted ligands and various derivatives will be needed.

In summary, the presence of a pronounced line at 503 cm^{-1}, attributable to S–S stretching, in the RR spectrum of the naturally-occurring green form of BCD provides support for the view that CoA persulphide is the charge-transfer donor in this complex. The other lines in the spectrum suggest that the electrons are shared with the N(5) and C(4a) atoms of the isoalloxazine ring. The presence of an acyl substituent in acetoacetyl-CoA, rather than an extra sulphur atom in CoA persulphide, apparently results in perturbation of several other atoms on the isoalloxazine structure. It will be of interest to study the RR spectrum of some of the other charge-transfer complexes of BCD that have been characterised [6,19].

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