PRELIMINARY NOTES

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Energy-dependent protein conformational transitions in mitochondrial membranes

Since the functional characteristics of mitochondria in different respiratory states were described, various investigations have been directed towards the analysis of the gross morphological changes which accompany the transitions between these states[1]. We have attempted, therefore, to correlate these changes with transitions at the molecular level. Previously we have shown by solid-state infrared spectroscopy that the membrane proteins of rat-liver mitochondria[2], unlike those of plasma membranes[3], have a significant proportion of their peptide linkages in the anti-parallel β-conformation. We now show that this proportion depends upon the metabolic state of the mitochondria.

Rat-liver mitochondria[4] were osmotically ruptured by washing 3 times in 7 mM phosphate buffer (pH 7.4)[5] to reduce spectral interference by soluble mitochondrial substances. Such mitochondrial membranes are fully capable of supporting electron transport but control of respiration by phosphorylation is only about half as effective as in intact mitochondria[6].

25 μl of a suspension of lysed mitochondria (1–2 mg protein per ml) were applied to AgCl discs to form a rectangular film. The suspensions were "fixed" by immersion in liquid N_2 and the frozen films lyophilized for subsequent infrared spectroscopy.

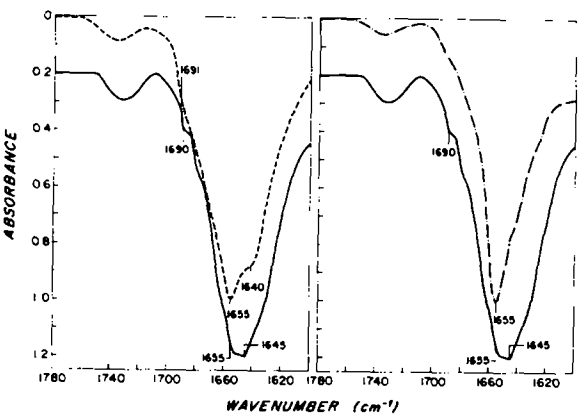

Fig. 1. Infrared spectra of lysed mitochondria to show State I–State IV transitions. 25 μl lysed rat-liver mitochondria in 0.15 M KCl–7 mM phosphate buffer (pH 7.4) were frozen at −180° after incubation at 25°, with (a) 15 μl buffered KCl for 1 min (– – – –); (b) 10 μl buffered KCl and 5 μl 0.8 mM succinate for 1 min (———); (c) 5 μl buffered KCl and 5 μl 0.8 mM succinate for 1 min, followed by 5 μl 0.8 mM KCN for 0.5 min (–·—·–). The two curves in each panel are displaced by 0.2 absorbance unit. All additions made in buffered KCl.

A Perkin–Elmer Model 221 infrared spectrophotometer was used to determine spectral changes in the Amide I (coupled C=O stretching) region of the infrared spectrum.

In Fig. 1 we show the infrared spectrum of freshly prepared, lysed mitochondria lyophilized from Respiratory State I (excess O_2; no added substrate or ADP). A band near 1655 cm^{-1} reflects the presence of peptide in the "unordered" or α-helical conformations, or both[7]. The shoulder between 1630 and 1645 cm^{-1} and the inflection near 1690 cm^{-1} arise from the peptide in the anti-parallel β-form.

We promoted the mitochondria into Respiratory State IV (excess O_2 and substrate; no added ADP) by incubation in the presence of 0.1 mM succinate before freezing and lyophilizing. As shown in Fig. 1, this produces a substantial increase in the absorbance at 1630–1645 cm^{-1}, relative to that observed in State I. In addition, the inflection at 1690 cm^{-1} becomes more prominent. The response to succinate is abolished in the presence of 100 μM CN^-.

To induce Respiratory State III (excess O_2, substrate and ADP), we added ADP to State IV mitochondria (final concn., 0.25 mM) before freezing and lyophilizing.

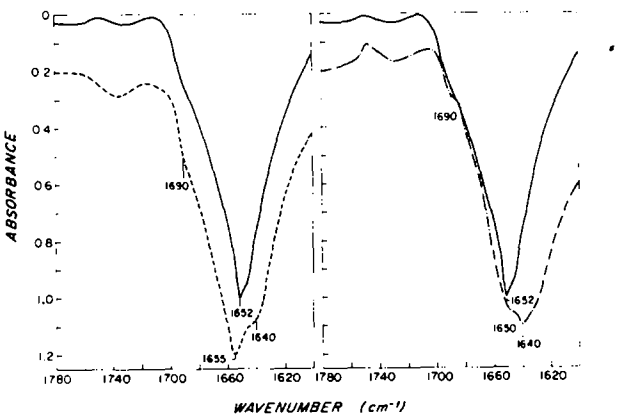

Fig. 2. Infrared spectra of lysed mitochondria to show State IV–State III transitions. 25 μl lysed rat-liver mitochondria in 0.15 M KCl–7 mM phosphate buffer (pH 7.4) were frozen at −180° after incubation at 25° with (a) 15 μl buffered KCl for 1 min (– – – –); (b) 5 μl buffered KCl and 5 μl 0.8 mM succinate for 1 min, followed by 5 μl 2.0 mM ADP for 0.5 min (———); (c) 5 μl 0.8 mM succinate and 5 μl 0.8 mM 2,6-dinitrophenol for 1 min, followed by 5 μl 2.0 mM ADP for 0.5 min (–·—·–). The two curves in each panel are displaced by 0.2 absorbance unit. All additions made in buffered KCl.

As shown in Fig. 2, this produces a marked reduction in the absorbance below 1650 cm^{-1} relative to that in State I, and the inflection at 1690 cm^{-1} is considerably diminished. Another characteristic feature of the State III infrared spectrum is a sharpening of the band at 1650 cm^{-1}. When electron transport is uncoupled from phosphorylation by adding 2,4-dinitrophenol (0.1 mM), these manifestations of the State IV–State III transitions are not observed. The infrared spectrum of the peptide of mitochondrial membranes in the presence of succinate, ADP and 2,6-dinitrophenol is characterized by a broad band between 1630 and 1645 cm^{-1} (Fig. 2) with a shoulder at 1650–1655 cm^{-1}. The 1690 cm^{-1} inflection is very prominent. These features indicate a large increase in the proportion of membrane peptide in the anti-parallel β-conformation, apparently a reflection of the high level of uncoupled electron transport.

AgCl discs supporting dried films of 5 μl 2.0 mM ADP and 5 μl 0.8 mM 2,6-dinitrophenol were used in the reference path when necessary. At the concentration used succinate produced no absorbance increase in the Amide I region; ADP and 2,6-dinitrophenol caused a small increase in absorbance (*c* 0.02 absorbance unit) below 1670 cm^{-1} which then remained constant in the Amide I region.

One explanation of the observed spectral changes is that the proportion of β-conformation in mitochondrial proteins varies with the energy flux in the electron-transport and oxidative-phosphorylation paths. Alternatively, it is possible that desiccation of the membranes favors transition of some peptide into the anti-parallel β-conformation. However, infrared spectroscopy of lysed mitochondria in ^2H_2O suspension has confirmed the presence of protein in the β-conformation. In addition, dry films of plasma and ergastoplasmic membranes from various sources do not normally show evidence of β-conformation[3]. Furthermore, if lyophilized mitochondria are slowly rehydrated, they exhibit identical 2,6-dinitrophenol-stimulated ATPase activity to control mitochondria, indicating that lyophilization does not irreversibly uncouple phosphorylation from respiration.

The observed spectral changes could reflect the behavior of one or more mitochondrial membranes and/or matrical proteins. However, osmotic lysis elutes a large proportion of the latter[8]. We therefore suspect that State I–State IV and State IV–State III transitions involve reversible changes of secondary structure in some mitochondrial membrane proteins. One can conjecture that these proteins possess peptide regions which can fold into (and out of) the β-structure with certain metabolic transitions. In the absence of electron transport, these peptide regions would be predominantly in "unordered" and/or helical array. In the presence of electron transport, but in the absence of coupled phosphorylation, the labile peptide regions are promoted into the anti-parallel β-conformation. Initiation of phosphorylation, however, favors a decrease in the percentage of protein in the β-conformation.

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