MAGNETIC CIRCULAR DICHROISM AND MAGNETOOPTICAL ROTATORY DISPERSION OF SUBMITOCHONDRIAL PARTICLES AT ROOM AND LIQUID NITROGEN TEMPERATURES

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1. Introduction

Magnetic optical activity methods – magneto-optical rotatory dispersion (MORD) and magnetic circular dichroism (MCD) [1] – proved to be a useful tool in studies of certain heme proteins, such as myoglobin, hemoglobin [2–4], cytochrome *c* [5–7], b_2 [8,9], b_5 [9] and also in experiments with a model systems [10–12]. MORD and MCD are sensitive to heme iron oxidoreduction transitions as well as to heme surrounding and the nature of axial ligands in heme proteins [4,11,12]. High resolution power of these methods provides an advanced opportunity for investigation of multienzyme systems, which cannot be resolved into individual components without a concomitant loss of activity and/or native structure of these components. Mitochondrial respiratory chain belongs to this type of integral system. Difference absorption spectroscopy [13] is one of most commonly used optical methods in studies of cytochrome components of mitochondria or submitochondrial particles (SMP). However, the individual absorption bands of several *b*-type cytochromes and cytochromes ($c + c_1$) present in respiratory chain are overlapped significantly in α-, β- and γ-regions and can not be well resolved even in low temperature spectrophotometric measurements.

We now want to demonstrate here the advantage of MORD and MCD methods when applied to the studies of SMP cytochrome chain. It will be shown that MCD and MORD effects arising from reduced *b*- and *c*-type cytochromes are almost completely separated at room temperature already, so that reduction state of these components may be easily followed quite free of mutual interference, resolution being further improved at liquid nitrogen temperature measurements. Recently C. Djerassi and coworkers [14] have reported of their MCD studies of cytochromes *P*-450 and b_2 in microsomal suspension.

2. Materials and methods

Phosphorylating beef heart submitochondrial particles were prepared essentially as described by Hansen and Smith [15]. Incubation medium contained Tris–HCl buffer 50 mM pH=7.4, sucrose 0.25 M, $MgSO_4$ 5 mM, rotenone 5 mM and uncoupler carbonyl cyanide *m*-chlorophenyl hydrasone (USSR) 1 μM. Protein was determined by biuret method.

MORD was measured at an automatic magneto-spectropolarimeter specially constructed as described previously [16]. MCD spectra were taken at a modified 'Roussel–Jouan' dichrograph equipped with electromagnet. Magnetic field direction was parallel or antiparallel to that of light propagation in the cases of MCD and MORD measurements, respectively, with respective field strengths of 13.4 and 7.5 kgauss. The signs of effects were considered as those in natural optical activity. Special cryostat was constructed for low temperature MCD measurements. Difference absorption spectra were recorded at a split beam/dual wavelength spectrophotometer with integrating sphere. The possibility of artifacts due to the turbidity of the sample in MCD studies have been discussed by

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several authors [17–19]. Though different scattering may be expected for left and right circular polarized light on the theoretical grounds, the difference is small and would not lead to significant errors. Artifacts due to concentration effects were found to be negligible in CD studies of the Soret region of SMP cytochromes [20] and should be even considerably less in our measurements in α- and β-regions of cytochromes absorption.

3. Results and discussion

MORD curves and MCD spectra of submitochondrial particles measured at room temperature are presented in fig. 1 and fig. 2 respectively. Difference absorption spectra (reduced minus oxidized) are drawn in the lower part of fig. 1. No correction was made for natural optical activity which was found to be negligible. Oxidized particles do not show any pronounced effects (dotted lines, figs. 1 and 2). When SMP are brought to anaerobiosis with ascorbate + TMPD (*N,N,N',N'*-tetramethyl-*p*-phenylen-diamine) as substrates two sharp minima appear on the MORD curve (dashed line, fig. 1) at 552and 561 nm due to the reduction of cytochromes (c+c_1) and b. The vibrational structure may be also seen in the β-absorption band region resembling that of isolated ferrocytochrome c [5,6]. The intensity of the signal at 561 nm is further increased upon the addition of succinate (not shown) or dithionite (solid line, fig. 1), the minimum however retaining its symmetrical form and peak position, so that no appearance of long-wavelength species of cytochrome b could be observed. The effects due to reduced b and (c+c_1) cytochromes are impressively well resolved and can be followed quite independently. These effects observed are typical A-terms (for description of possible A,B and C-term effects see ref. [1]), which in MORD are symmetrical relative to transition frequency, their minima coinciding with absorption maxima. Sharp and intense A-term signal characteristic of heme complexes with strong axial ligands [11,12] suggest the ligands of this type to occupy the fifth and the sixth coordination positions of heme iron in at least one of cytochromes b in situ.

The striking feature of MORD curves of reduced SMP is the absense of any significant effects at the

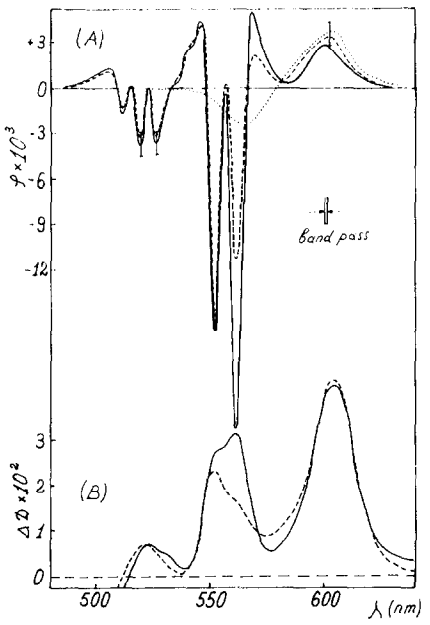

Fig. 1. MORD (A) and difference absorption spectra (B) of submitochondrial particles. A) SMP suspension in incubation medium, 24 mg protein/ml. Cuvette with optical path 2 mm. Room temperature. Dotted line – SMP oxidized in the presence of 5 mM of ferricyanide. Dashed line – particles made anaerobic with ascorbate (6 mM) + TMPD (300 μM) addition. Solid line – dithionite reduced particles. B) Conditions as in the legend to fig. 2.

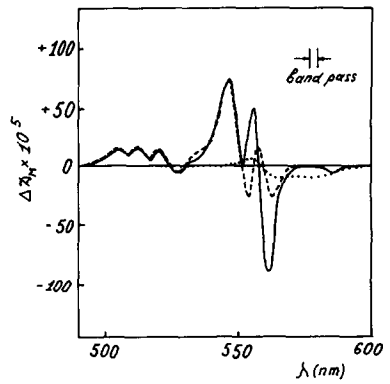

Fig. 2. MCD spectra of submitochondrial particles. SMP suspension (3.7 mg protein/ml) in incubation medium. Cuvette with optical path 10 mm. Room temperature. Dotted line – SMP oxidized aerobically. Dashed line – SMP made anaerobic with ascorbate (4 mM) + TMPD (200 μM). Solid line – dithionite reduced particles.

wavelength of cytochrome oxidase absorption maximum and also an apparent lack of cytochrome b_{565} (b_T) contribution to the minima at 561 nm. One should not however expect any direct correlations between absorbancy of the substance and its magnetooptical activity, the latter being by far more sensitive to the geometry of metal complexes in heme proteins.

In fig. 2 MCD spectra of the same preparation of SMP are shown. The same information is provided by MORD and MCD, the reciprocal recalculation of the curves being possible. Overlapping of reduced b and (c+c_1) cytochromes effects is however more pronounced visually in MCD, due to different shapes of MORD and MCD curves. Nevertheless short wavelength lobe of (c+c_1) and long wavelength lobe of b s-like curves are free from any mutual interference so that independent followings of cytochromes b and (c+c_1) reduction states is possible in MCD measurements too.

MCD spectrum of dithionite reduced SMP frozen at 80°K is shown in fig. 3. A significant narrowing of the peaks as well as intensification of the effects

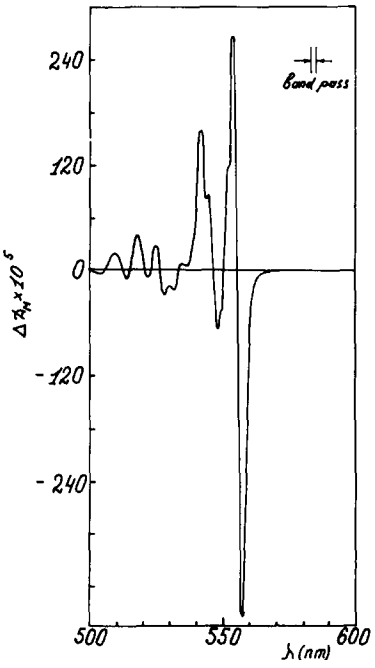

Fig. 3. MCD spectrum of reduced submitochondrial particles at 80°K. SMP suspension in the incubation medium was mixed with glycerol at 1:1 (v/v) ratio up to a final concentration of 24 mg protein/ml and reduced with dithionite. The cuvette with optical path of 1,5 mm.

provide a better resolution, thus some heterogenity of b and (c+c_1) signals may be observed. The spectrum is the mean of several readings and seems to reproduce properly the principal features of particles MCD properties despite the relatively high noise to signal ratio level. Besides the resolution improved an advantage may be taken out of low temperature MCD measurements, in trapping SMP at different physiological states. Results of our MORD and MCD studies of respiratory chain components of submitochondrial particles are to be published in more detail later on.

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