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The Induced Optical Rotatory Dispersion of Mercury Orange-Phosphoenolpyruvate Carboxylase*

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Synopsis. On reacting with a SH group, the mercury orange enzyme formed, showed a positive Cotton effect near 500 nm, and the optical activity of which was remarkably induced by adding ADP or PEP. It seemed that ADP or PEP might move to the vicinity of the active amino acid residues.

It has been reported that phosphoenolpyruvate carboxylase (EC4.1.1.a), which is activated by the manganous ion, has a sulfhydryl group in the active center, judging from the fact that the activity of this enzyme is inhibited by the addition of *p*-chloromercury benzoate.[1-3] A kinetic study of this enzyme has been carried out by us.[4] It is, however, necessary to accumulate more data for a better understanding of the active center of this enzyme.

It is known that 1-(4-chloromercuriphenylazo)naphthol-2 (mercury organge) reacts with enzyme to form mercury–sulfur bondings.[5,6] It has been found that phosphoenolpyruvate carboxylase reacts with mercury orange and that the product shows an absorption maximum near 492 nm because of the azochromophore of mercury orange. This chromophoric group at the active center of the enzyme has been found to be environmentally sensitive to the optical activity.

This paper will show how the environment of the active center of phosphoenolpyruvate carboxylase can be studied using mercury orange by means of the optical rotatory dispersion spectrophotometric method.

Experimental

The phosphoenolpyruvate carboxylase was prepared from Baker's yeast in the way described in a previous paper.[4] The optical rotatory dispersion (ORD) and the visible spectra were measured by means of the JASCO ORD/UV/CD-5 spectrometer. The enzyme and the substrates were dissolved into a 0.25 mol/l phosphate buffer solution (pH 6.0), and the ORD spectra were measured at 18 °C.

Results and Discussion

Mercury orange exhibits an absorption maximum at 500 nm and shoulders near 450 nm and near 550 nm on a visible spectrum, as is shown in Fig. 1. The shoulder near 450 nm and λ_{max} at 500 nm might be assigned to the *cis* and *trans* isomers respectively, for it is known that a diazo-containing compound gives *cis* and *trans* configurations upon transformation, and because the absorption maximum of the *cis* isomer generally appears at a shorter wavelength than that of the *trans* isomer.[7] The mercury orange-binding enzyme (MO–enzyme) shows an absorption spectrum similar to that of mercury orange. The absorbance near 500 nm decreases and the absorbance near 450 nm increases upon the addition of adenosine-5′-diphosphate (ADP) or phosphoenolpyruvate (PEP) into the MO-enzyme (Fig. 1). It could be said that ADP or PEP are located near the mercury orange group in the MO-enzyme and affect the transformation of a

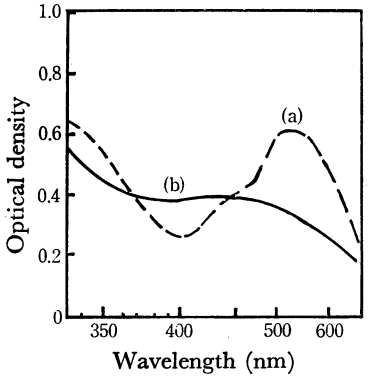

Fig. 1. Absorption spectra of mercury orange–phosphoenolpyruvate carboxylase complex.
a): MO–enzyme (1.0 mmol/l) and MO (1.0 mmol/l, pH 6.0)
b): MO–enzyme (1.0 mmol/l)+ADP (1.0 mmol/l) and MO–enzyme (1.0 mmol/l)+PEP (1.0 mmol/l)

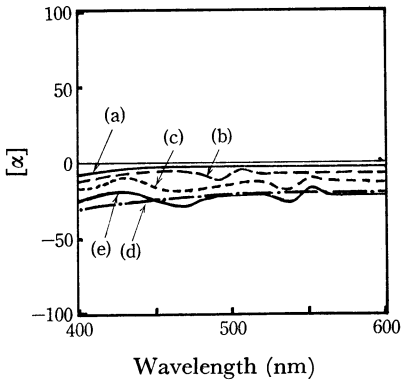

Fig. 2. ORD spectra of mercury orange–phosphoenolpyruvate carboxylase complex.
a): base line, manganous ion, bicarbonate ion, phosphoenolpyruvate and mercury orange
b): MO–enzyme (1.0 mmol/l), MO–enzyme (1.0 mmol/l)+Mn^{2+} (1.0 mmol) and MO–enzyme (1.0 mmol/l)+bicarbonate ion (1.0 mmol/l)
c): MO–enzyme ((1.0 mmol/l)+PEP (1.0 mmol/l)
d): ADP (1.0 mmol/l)
e): MO–enzyme (1.0 mmol/l)+ADP (1.0 mmol/l)

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diazo group of mercury orange.

The MO–enzyme showed a positive Cotton effect near 500 nm (Fig. 2). Neither mercury orange nor the enzyme exhibits an optical activity in the same region. The ORD spectrum of the MO–enzyme is not influenced by the addition of manganous ion (Fig. 2), which means that there is no interaction between the manganous ion and the active center of the enzyme. This is to be expected if mercury orange might be located in the active center, thus forming mercury-sulfur binding, and if the manganous ion should bind with the sulfhydryl group in the active center, thus activating the enzyme. In this connection, the bicarbonate ion was found not to interact with the MO–enzyme. The optical activity of the MO–enzyme is remarkably induced by adding ADP or PEP. It can be deduced from the visible absorption and the ORD spectra that the diazo group in the MO–enzyme has the *trans* configuration mainly, and that this configuration can be influenced by the interaction between the mercury orange goup and ADP or PEP. It seemed that ADP or PEP might move to the vicinity of the active site of the enzyme.

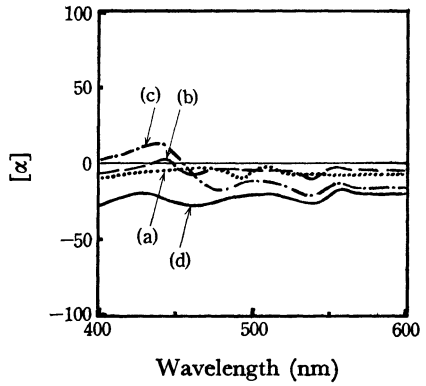
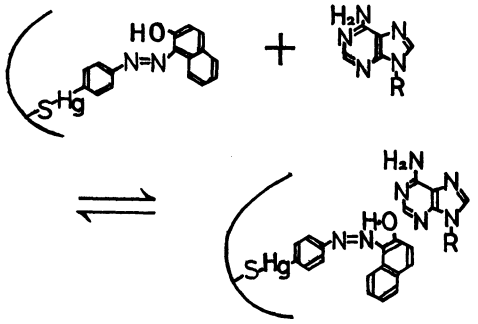


Fig. 3. ORD spectra of mercury orange–phosphoenolpyruvate carboxylase–adenine derivatives complex.
a): MO–enzyme (1.0 mmol/l)
b): a)+adenine (1.0 mmol/l)
c): a)+adenosine (1.0 mmol/l)
d): a)+ADP (1.0 mmol/l)

The optical activity of the MO–enzyme was induced by the addition of adenine, adenosine, or adenosine-5′-triphosphate (ATP) instead of ADP, as is shown in Fig. 3. The ORD spectrum of ATP seems almost the same as ADP, and those of adenine and adenosine exhibit a tendency similar to that of ADP. In every case, another Cotton effect appears around 400 nm. It seems that the adenine or adenine group of adenosine, ADP or ATP, may be located at the binding site of the active center, thus transform in the diazo group from the *trans* to the *cis* configuration as follows:



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