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INTERACTION BETWEEN BRAIN ENZYMES

GLUTAMATE DEHYDROGENASE AND ASPARTATE AMINOTRANSFERASE

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SUMMARY

Fluorescence spectroscopic methods were used to study the interaction between aspartate aminotransferase and glutamate dehydrogenase isolated from pig brain. The conversion of the P-pyridoxal form of the aminotransferase to the P-pyridoxamine form of the enzyme is easily monitored by recording emission spectra upon excitation at 330 nm. Evidence for the interaction between the enzymes was obtained from fluroescence measurements conducted on aspartate aminotransferase label with a fluorescence probe (1-5-AEDANS) attached to one sH residue of the protein. The interaction of the aminotransferase (1μM) with glutamate dehydrogenase (2μM) brings about an enhancement as well as a blue shift in the band position of the fluorescence emitted by the dansyl chromophore. Polarization of fluorescence measurements conducted over a wide range of temperatures reveal that the rotational correlation time of aspartate aminotransferase (35 n.seconds) is increased to a value of 100 n.seconds upon addition of glutamate dehydrogenase.

INTRODUCTION

The enzyme glutamate dehydrogenase has transaminase dehydrogenase activity, i.e., in the presence of NADH, NH_4^+ and aspartate aminotransferase, glutamate dehydrogenase catalyzes the conversion of the P-pyridoxal form of the aminotransferase to the P-pyridoxamine form (1).

In addition, it has been demonstrated that formation of a complex between the interaction enzymes facilitates the reaction of glutamate dehydrogenase with the P-pyridoxal form of the aminotransferase (2). The interaction occurs at concentrations of the two enzymes considerably lower than those known to exist in the mitochondrial matrix.

ABBREVIATIONS

1-5-I-AEDANS (N-Iodoacetylaminoethyl)-5-Napthylamine-1-sulfonic acid.

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Since the reaction between the two enzymes may play an important regulatory function, it was thought of interest to investigate whether glutamate dehydrogenase isolated from pig brain has transaminase dehydrogenase activity.

This communication reports the results obtained when the interaction between aspartate aminotransferase and glutamate dehydrogenase from pig brain is monitored by fluorescence spectroscopy.

EXPERIMENTAL PROCEDURES

Purification of the enzymes. Aspartate aminotransferase from pig brain was purified according to the procedure of Sizer and Jenkins (3) as modified by Martinez et al. (4). The purified enzyme has a molecular weight of 90.000 daltons as determined by "gradipore" electrophoresis (5) and Sephadex-G-200 gel filtration. Glutamate dehydrogenase from pig brain was purified by a combination of ion exchange chromatography and blue-dextran-sepharose chromatography (to be published). The enzyme preparation migrates as a single protein and activity band on analytical gel electrophoresis.

The enzymes tend to form high molecular weight polymers at concentrations larger than 1 mg/ml. The molecular weight of the monomer is approximately 300.000 daltons.

Labelling of the transaminase. Aspartate aminotransferase at a concentration of 5 mg/ml was allowed to react with the reagent 1-5-I-AEDANS (1 mM) at pH 7.4, 4°C. The reaction was allowed to proceed for 20 hours at 4°C. Excess of free reagent was removed by dialysis against 0.1M phosphate (pH 7.4), followed by gel filtration through Sephadex-G-25. The degree of labelling of the enzyme was determined spectrophotometrically using an extinction coefficient of 6.2 $10^3 M^{-1} cm^{-1}$ at 340 nm (6).

Spectroscopy. Fluorescence and polarization of fluorescence measurements were performed in instruments designed in our laboratory (7). For polarization of fluorescence measurements the excitation was set at 340 nm, and the emission was filtered through a glass filter (C-S-3-u2, Corning glass). Fluorescence decay measurements were performed in an Ortec model 9200 nanosecond spectrometer. Time-base calibration of the instrument was performed using a solution of quinine sulphate, whose lifetime is 19.5 ns. The excitation was set at 340nm, and the emission was filtered through a glass filter (C-S-3-72, Corning glass). Fluorescence decay times were corrected for the finite duration of the exciting light pulse using the method of Ware et al (8). Absorption spectra were recorded in a Cary, model 15, spectrophotometer.

RESULTS AND DISCUSSION

In the presence of NADH and NH_4HCO_3, glutamate dehydrogenase catalyzes the conversion of the enzyme aspartate aminotransferase from the P-pyridoxal to the P-pyridoxamine form. This reaction is easily monitored by recording

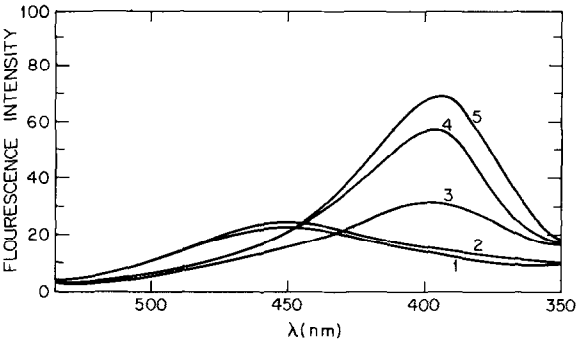

Figure 1

Emission spectra of NADH (4μM) + $(NH_4)HCO_3$ (0.0125M) (1); NADH (4μM) + NH_4HCO_3 (0.0125M) + transaminase (1μM) at pH 7.4 (2); NADH (4μM) + NH_4HCO_3 (0.0125M) + transaminase (1μM) + glutamate dehydrogenase (2μM) at pH 7.4 (3). The same reaction mixture after addition of H_2KPO_4 (0.5M), preincubated 30 minutes at 25°C (4). The same reaction mixture after addition of H_2KPO_4 preincubated for 1 hour at 25°C (5). Excitation wavelength 330 nm.

the emission spectra of the reaction mixture over the spectral range from 340 to 550 nm upon excitation with light of 330 nm. The results of such measurements are included in Figure 1, where it may be seen that the addition of glutamate dehydrogenase to the reaction mixture containing NADH, holo-transaminase (P-pyridoxal form) and NH_4HCO_3, induces in parallel to a decrease in the intensity of the fluorescence band due to NADH, the appearance of a new emission band centered at around 395 nm. The latter emission band has all the characteristics of P-pyridoxamine bound to the aminotransferse. If this interpretation is correct, then the dissociation of the cofactor P-pyridoxamine from the aminotransferase would result in fluorescence yield enhancement (9). As shown in Figure 1, the dissociation of the cofactor P-pyridoxamine from the active site of the aminotransferase upon decreasing the pH of the incubation mixture from 7.4 to 5, brings about an increase in the intensity of the fluorescence band centered at 395 nm.

Thus, conversion of aspartate aminotransferase from the P-pyridoxal to the P-pyridoxamine form takes place when NADH is oxidized to NAD^+ in the

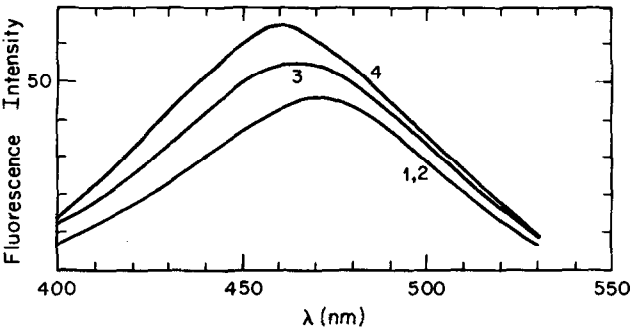

Figure 2

Emission spectra of aspartate aminotransferase-1-5-AEDANS (1μM) in the absence (1) and presence of bovine serum albumin (2μM) (2) and in the presence of L-aspartate (11mM) + oxaloacetate (1mM) at pH 7.4 (3). Emission spectra of the mixture aspartate aminotransferase-1-5-AEDANS (1μM) + glutamate dehydrogenase (2μM) at pH 7.4 (4). Excitation wavelength 360 nm.

presence of glutamate dehydrogenase. It should be noted that the concentration of NADH (4μM) is comparable in magnitude to the concentrations of the enzymes aspartate aminotransferase (1μM) and glutamate dehydrogenase (2μM).

Interactions Between Enzymes.

In order to detect the formation of a macromolecular complex between the interacting enzymes, the fluorescence properties of aspartate aminotransferase tagged with a dansyl fluorescence probe were examined in the presence and absence of glutamate dehydrogenase. The chemically accessible thiol groups of aspartate aminotransferase were allowed to react with 1-5-I-AEDANS. The enzyme binds 1 mole of the dansylated derivative without any loss of catalytical activity. The labelled enzyme exhibits an emmission band at around 470 nm when excited at either 335 or 365 nm.

The fluorescence emitted by the molecule of 1-5-I-AEDANS covalently bound to a thiol group decays in a monoexponential manner with a decay time of 12.5 nanoseconds.

The intensity as well as the band position of the dansyl fluorescence is influenced by ligands which induce conformational changes in the protein.

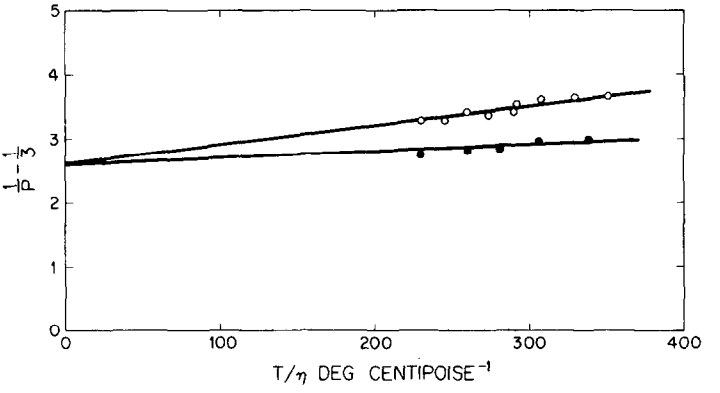

Figure 3

Perrin's plot for aspartate aminotransferase-1-5-AEDANS (1μM) (o) and aspartate aminotransferase-1-5-AEDANS (1μM) + glutamate dehydrogenase (2μM) (●) at pH 7.4. Excitation wavelength 340 nm.

As shown in Figure 2, the addition of the substrate pair L-aspartate, oxaloacetate to the dansylated enzyme causes a small blue shift in the band position of the emission spectra. Similar spectral changes, i.e. enhancement of the dansyl fluorescence and change in band position, are observed when aspartate aminotransferase (1μM) is mixed with glutamate dehydrogenase at a concentration of 2μM. These results are interpreted to mean that the binding of aspartate aminotransferase to glutamate dehydrogenase results in a perturbation of the microenvironment surrounding the dansyl fluorophore. In marked contrast to the effect observed with glutamate dehydrogenase, other enzymes tested, i.e., lactic dehydrogenase, aldolase, lysozyme, alcohol dehydrogenase, failed to perturb the emission band of the dansylated protein.

Further support for the contention that glutamate dehydrogenase and aspartate aminotransferase from pig brain tend to aggregate in solution, was derived from polarization of fluorescence measurements conducted over a wide range of temperatures.

When the polarization of fluorescence properties of the dansylated

aminotransferase were examined over the temperature range 5-30°C, it was found that the plot 1/P - 1/3 vs T/n is linear within experimental error and fits Perrin's equation for spherical macromolecules. Taking τ, the fluorescence lifetime of the chromophore to be 12.5 n.s, the rotational correlation time for the aminotransferase is 35 n.s when calculated by means of equation (1).

$$\phi = \frac{\tau \cdot \frac{1}{P_0} - \frac{1}{3}}{\frac{1}{P} - \frac{1}{P_0}} \qquad (1)$$

In the presence of glutamate dehydrogenase, the degree of fluorescence polarization changes in the manner depicted in Figure 3. The apparent rotational correlation time increases to a value of 100 n.s.

The increase in the rotational correlation time of the amino-transferase is attributed to the formation of stable macromolecular complex with glutamate dehydrogenase.

ACKNOWLEDGEMENTS

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