THE ULTRAVIOLET FLUORESCENCE SPECTRA OF DPN-LINKED ISOCITRATE DEHYDROGENASE FROM BOVINE HEART

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SUMMARY

The emission maximum of DPN-linked isocitrate dehydrogenase from bovine heart shifted from 316 nm to 324 nm as the excitation wavelength was varied from 265 nm to 300 nm. This shift was accompanied by a nonproportional change in fluorescence intensity. Comparisons of the emission spectra of model compounds in aqueous buffer at pH 7.07 and n-butanol showed that lowered solvent polarity led to a blue shift of the peak of free tryptophan without significant change of fluorescence intensity, whereas the fluorescence intensity of tyrosine amide increased markedly without change in emission maximum. The emission peak of mixtures of tryptophan and tyrosine amide shifted to shorter wavelengths as the proportion of tyrosine amide increased. The results suggest a major contribution of tyrosine to the overall fluorescence of the dehydrogenase. DPNH caused quenching and a blue shift of the protein fluorescence maximum when excited between 270 nm and 290 nm, indicating that the two tryptophan residues per subunit of enzyme are located in different microenvironments of the protein and that DPNH may interact preferentially with the residue emitting at the longer wavelength.

In recent reports on DPN-linked isocitrate dehydrogenase we have shown that the emission maximum of the enzyme is at 317 nm when excited at 280 nm (1, 2). Since tryptophan is the major fluorescent species in proteins, the 30 nm blue shift of the emission peak of the enzyme from that of free tryptophan in water at 347 nm suggests the possibilities that the two tryptophyl residues (2) of this enzyme are located in a rather hydrophobic region and that the tryrosyl residues contribute to the overall fluorescence. It was also observed that quenching of protein fluorescence by 8-anilinonaphthalene sulfonate (ANS) was accompanied by a 7 nm blue shift, indicating that ANS interacts differently with each of the two tryptophyl groups per subunit of the enzyme (2).

In this communication, we have analyzed further the fluorescence spectra of DPN-linked isocitrate dehydrogenase as affected by variations of excitation wavelengths and by quenching of protein fluorescence by interaction with DPNH.

METHODS

DPN-linked isocitrate dehydrogenase from bovine heart was purified, assayed and stored in the same way as described previously (3, 4, 5).

Fluorescence was measured in the apparatus described previously (4). The values of Chen (6) for the absolute quantum yields of tyrosine amide and tryptophan in water were used for relative comparisons.

Fluorescence quenching by DPNH - The emission spectra of 6.5 μM enzyme solutions in 0.1 M sodium Hepes at 25° excited over the range 270 nm to 290 nm were measured after addition of increasing concentrations of DPNH (0 to 18 μM). The spectra reported were corrected for light intensity and background.

RESULTS AND DISCUSSIONS

Dependence of Emission Spectra of DPN-linked Isocitrate Dehydrogenase on Excitation Wavelengths: It has been found that the relative fluorescence of tryptophan or tyrosine depends on the excitation wavelength (7). Between 265 nm and 290 nm isocitrate dehydrogenase in 0.1 M Na-Hepes at pH 7.07 showed a much larger excitation wavelength dependent change in fluorescence intensity than solutions of tryptophan in 0.1 M Na-Hepes or in n-butanol; the wavelength dependent changes in fluorescence intensity of the enzyme corresponded more closely to those of solutions of tyrosine amide in 0.1 M Na-Hepes buffer or in n-butanol (Figure 1). The emission maximum of the enzyme shifted from 316 nm to 324 nm as the excitation wavelength was varied from 265 nm to 300 nm; however, the emission maximum of each of the free amino acids remained unchanged (Figure 1 insert). These results indicate that the tyrosyl residues of the enzyme could make an important contribution to the overall fluorescence of the protein. Similar observations have been reported for papain (7) and growth hormone (8) when excited at shorter wavelengths. However, an unequivocal demonstration of the contribution of tyrosyl residues to protein fluorescence was not possible since the emission maximum of tyrosine at 303 nm could not be detected as a peak or shoulder in the emission spectrum of isocitrate dehydrogenase.

In an attempt to resolve the relative contributions of these aromatic amino acids to protein fluorescence, the emission spectra of mixtures of free tyrosine amide and tryptophan dissolved in 0.1 M sodium Hepes at pH 7.07 or in n-butanol were examined at an excitation wavelength of 280 nm. In 0.1 M

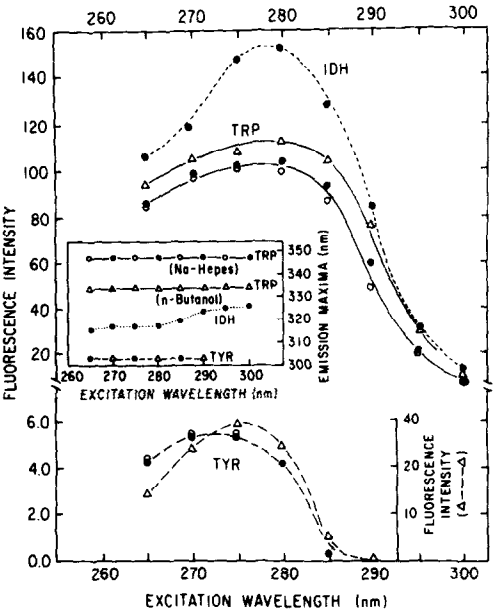

Figure 1. Comparison of emission properties as a function of excitation wavelength of DPN-linked isocitrate dehydrogenase (IDH), tryptophan (TRP) and tyrosine amide (TYR) in various solvents. The fluorescence intensities reported were measured at the emission maxima. Fluorescence intensity scale on left: Tryptophan in n-butanol (—Δ—), 0.1 M Na-Hepes at pH 7.5 (—O—), and 0.1 M Na-Hepes at pH 6.5 (—●—). Isocitrate dehydrogenase in 0.1 M Na--Hepes at pH 7.07 (·····●·····). Tyrosine amide in 0.1 M Na-Hepes at pH 7.5 (---O---) and 0.1 M Na-Hepes at pH 6.5 (---●---). Fluorescence intensity scale on right: Tyrosine amide in n-butanol (---Δ---). Insert: Positions of emission maxima of the substances in the solvents above as a function of excitation wavelength.

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sodium Hepes the emission spectra of tyrosine amide and tryptophan were resolved clearly with emission maxima at 303 nm and 347 nm, respectively (Fig. 2A). A change of the solvent system from Hepes buffer to n-butanol (Fig. 2B) resulted in a shift of the tryptophan emission maximum from 347 nm to 334 nm without a significant change of fluorescence intensity, whereas the fluorescence intensity of tyrosine amide increased over 5-fold without a shift in the emission maximum. The major emission maximum of mixtures of the amino acids shifted to shorter wavelengths as the ratios of tyrosine amide to tryptophan increased (Fig. 2); in Hepes buffer the emission maximum (347 nm) for tryptophan alone, was gradually shifted to the blue with increasing proportions of tyrosine amide in the mixture. Energy transfer due to interaction

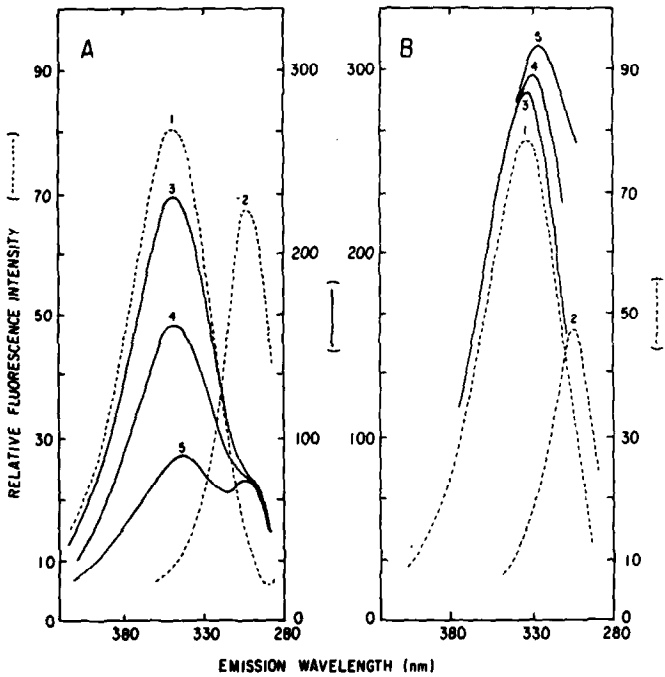

Figure 2. The emission spectra of mixtures of tryptophan and tyrosine amide in solutions:

(A) Emission spectra in 0.1 M sodium Hepes at pH 7.07: (1) 3.3 μM tryptophan, (2) 33.3 μM tyrosine amide, (3) 10 μM tryptophan and 33.3 μM tyrosine amide, (4) 6.7 μM tryptophan and 33.3 μM tyrosine amide, (5) 3.3 μM tryptophan and 33.3 μM tyrosine amide.

(B) Emission spectra in n-butanol: (1) 3.3 μM tryptophan, (2) 3.3 μM tyrosine amide, (3) 10 μM tryptophan and 3.3 μM tyrosine amide, (4) 10 μM tryptophan and 10 μM tyrosine amide, (5) 10 μM tryptophan and 16.7 μM tyrosine amide.

between the amino acids did not occur since it could be calculated that the sum of the contributions to fluorescence of tyrosine amide and tryptophan to the mixtures accounted for the overall fluorescence yield.

The above results suggest that the inability to resolve the contributions of tyrosyl and tryptophyl residues to the emission spectra of the enzyme could be attributable to a combination of effects, i.e., the large enhancement of fluorescence yield of tyrosine amide and the blue shift of tryptophan emission maxima in an environment of lower polarity. The emission maximum of isocitrate dehydrogenase is at a considerably shorter wavelength than that of free tryptophan in water. This is consistent with location of these aromatic amino acids

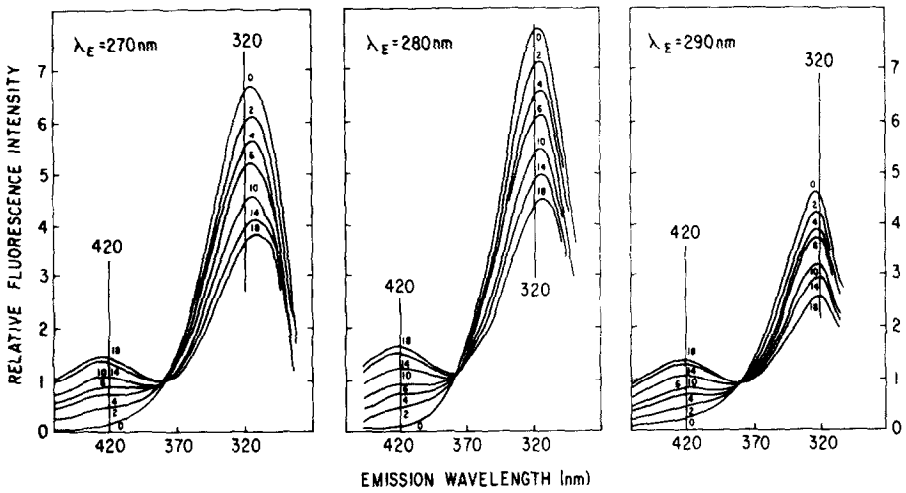

Figure 3. The effect of DPNH on fluorescence spectra of DPN-linked isocitrate dehydrogenase excited at 270 nm, 280 nm, and 290 nm. Enzyme solution (6.5 μM) in 0.1 M Na-Hepes at pH 7.07 and 25° was present in all measurements. The concentrations of DPNH varied as 0 μM, 2 μM, 4 μM, 6 μM, 10 μM, 14 μM and 18 μM.

in regions of low polarity on the enzyme.

Fluorescence Quenching by DPNH: It was demonstrated in previous reports that the binding of DPNH to the enzyme led to quenching of protein fluorescence, enhancement of DPNH fluorescence and energy transfer from enzyme to DPNH (9). In addition, the present investigations have shown that binding of DPNH is also accompanied by changes in position of the protein emission peak (Fig. 3). The effects of the ligand on protein emission were studied by adding increasing amounts of DPNH (0 to 18 μM) to 6.5 μM isocitrate dehydrogenase. When excited at 280 nm, quenching of protein fluorescence by high concentrations of DPNH was accompanied by a 7 nm blue shift of the protein emission maximum (from 317 nm to 310 nm). The spectra were very similar to results obtained previously on the interaction of enzyme with ANS (1, 2). When excited at 270 nm, a distinct shoulder at 303 nm started to appear as DPNH concentration was increased and protein fluorescence was quenched. These results are consistent with the proposal that tyrosine contributes to the overall fluorescence of the enzyme and reflect the preferred energy transfer from tryptophan to DPNH (9, 10). When

excited at 290 nm, where tyrosine fluorescence is nearly negligible, quenching of protein fluorescence was accompanied by a small blue shift (from 324 nm to 319 nm). These results suggest that DPNH interacts differently with each of the two tryptophyl residues of the enzyme. The blue shift indicates that DPNH may preferentially interact with the residue with a higher emission maximum (close to or higher than 324 nm). The other tryptophyl residue with a emission maximum near or lower than 319 nm is likely to be located in a less polar region than the one which interacts with DPNH. A similar conclusion was reached by Purkey and Galley in experiments with horse liver alcohol dehydrogenase (11).

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