Contact Versus Energy Transfer Fluorescence Quenching in the Sulfur Substituted Form of the Enzyme Rhodanese: A Study Using Cesium Ion Resolved Emission Spectra

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Received September 23,1975

Summary

The intrinsic fluorescence of the enzyme rhodanese (EC 2.8.1.1) can be resolved into separate contributions from solvent accessible and solvent inaccessible tryptophan residues by comparing spectra run in 2 M NaCl with those run in the quenching solution, 2 M CsCl. Both these classes of tryptophan residues are quenched when sulfur is transferred to rhodanese forming a sulfur substituted enzyme which is an intermediate in the catalytic cycle. This observation is consistent with a non-radiative energy transfer mechanism for quenching as opposed to a mechanism requiring direct contact between the bound sulfur and an active site tryptophan. Therefore, the data supports the hypothesis that the primary stabilizing influence in forming the substituted enzyme intermediate is a persulfide bond between an active site sulfhydryl group and the transferred sulfur.

Introduction

The enzyme rhodanese (EC 2.8.1.1) cycles between free and sulfur substituted forms as it catalyzes the transfer of the outer sulfur of thiosulfate to a nucleophilic acceptor (1). Conclusions as to the mode of sulfur binding have been drawn in part from the fluorescence characteristics of the enzyme. It has been observed that when sulfur is transferred to the enzyme there is a significant quenching of the tryptophan residue fluorescence which can be relieved on sulfur removal by sulfite or cyanide (2). This quenching was originally attributed to a direct interaction of the bound sulfur with a tryptophan residue

presumed to be present at the active site (2). Recent studies (3), on the other hand, have proposed that the observed quenching arises from non-radiative energy transfer due to spectral overlap between tryptophan emission and the absorption of a persulfide intermediate formed between an essential enzymic sulfhydryl group (4) and the transferred sulfur. It has been noted, however, that this suggested enzymic persulfide does not display the stability or extinction coefficient of typical small molecule persulfides (1).

The present study has been undertaken to distinguish these two possible mechanisms. To this end we have investigated the catalytically correlated quenching using cesium ion to resolve the rhodanese fluorescence spectrum.

Materials and Methods

Bovine liver rhodanese (M.W. 32,600) was prepared by the method of Horowitz and DeToma (5) with minor modifications and stored at -70°C as an ammonium sulfate suspension. All other materials were the best available commercial grades and were used as obtained.

Enzyme activity was assayed by the method of Wang and Volini (6). Protein concentrations were measured with a modified biuret procedure (7). Fluorescence measurements were made with a fluorometer constructed in this laboratory. Excitation was at 290 nm and emission spectra were scanned from 300-400 nm. The emission spectra were corrected using a set of factors obtained by comparing an emission spectrum of bovine serum albumin recorded on our instrument with a corrected spectrum.

Fluorescence quentum yields were calculated by comparing the areas under corrected spectra of rhodanese, bovine serum

albumin (Q = 0.39)[1] and β-lactoglobulin (Q = 0.12) solutions of equal optical densities.

Stock enzyme solution was prepared by incubating rhodanese (28 mg/ml, .58 IU/μg) in 0.16 M Tris buffer pH 8.6 with an approximate 100-fold molar excess of $Na_2S_2O_3$ to produce the sulfur-substituted form of the enzyme. After 10 min, the enzyme was desalted on a Bio-Rad P-2 column equilibrated with 0.16 M Tris pH 8.6 and eluted with the same buffer.

Samples for spectra were prepared by adding 0.5 ml of a diluted stock enzyme solution to either 2.0 ml of a 2.5 M salt solution in 0.16 M Tris buffer pH 8.6, or 2.0 ml of the buffer solution alone. These solutions also contained a 20-fold molar excess of $Na_2S_2O_3$. The final enzyme concentration was 0.07 mg/ml. After each spectrum was run, a 400-fold molar excess of Na_2SO_3 was added to produce the sulfur-free form of the enzyme, and the spectrum was immediately rerun.

Results and Discussion

Spectra of the sulfur-substituted form of rhodanese are shown in Figure 1. Curve I is the spectrum of enzyme in 2 M NaCl and with the exception of a somewhat higher quantum yield is virtually identical to the spectrum in buffer alone. The λ_{max} occurs at 332 nm. Curve II is the spectrum run in 2 M CsCl. The quantum yield is reduced due to cesium ion quenching of solvent accessible tryptophans (8). The λ_{max} is shifted to 324 nm because of a much larger contribution from internal, solvent inaccessible tryptophans. Curve III shows the difference spectrum, (Curve I - Curve II). Curve III is the spectrum of solvent

[1] abbreviations: Q, quantum yield; λ_{max}, emission maximum; E, unsubstituted form of rhodanese; ES, sulfur substituted form of rhodanese.

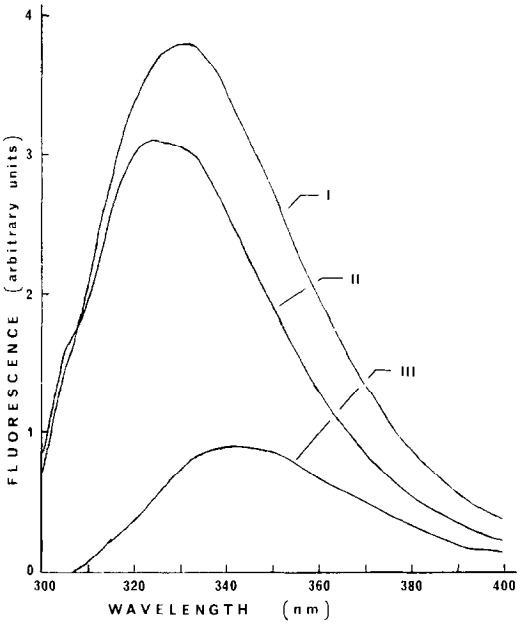

Figure 1. Corrected fluorescence emission spectra of sulfur substituted rhodanese in salt solutions. Curve I, sulfur-substituted rhodanese in 2 M NaCl; Curve II, sulfur-substituted rhodanese in 2 M CsCl; Curve III, difference between Curve I and Curve II. See the text for a detailed description of the sample preparation.

accessible tryptophans and has a λ_{max} at 340 nm (Burstein Class II, reference 9). Curves II and III, then, represent the partial resolution of the direct rhodanese spectrum into the contributions from solvent accessible and solvent inaccessible tryptophan residues.

Figure 2 compares the resolved spectra of rhodanese in the free and sulfur-substituted forms. Interestingly, there is no shift in the λ_{max} in any spectrum on conversion of one form to the other and both the solvent accessible and the buried residues

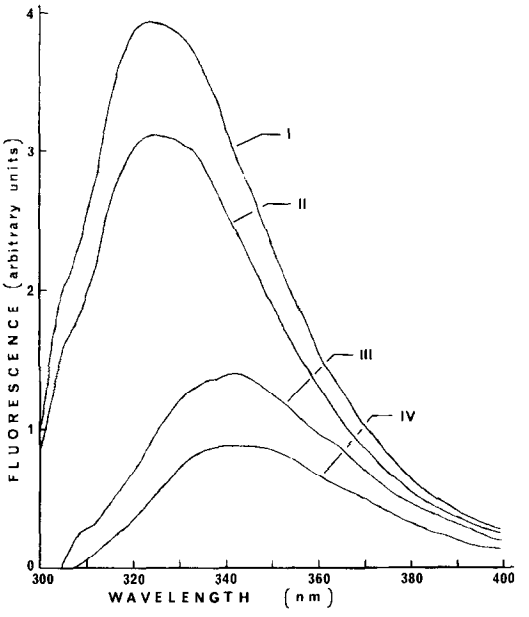

Figure 2. Corrected resolved fluorescence emission spectra of sulfur substituted and sulfur free rhodanese in salt solutions. Curve I, sulfur-free rhodanese in 2 M CsCl; Curve II, same as Curve II in Figure 1; Curve III, difference between a spectrum of sulfur-free rhodanese in 2 M NaCl and Curve I; Curve IV, same as Curve III in Figure 1. See the text for a detailed description of the sample preparation.

are quenched when the enzyme binds sulfur.

Pertinent fluorescence data for rhodanese is collected in Table I.

If the quenching was due entirely to contact between the substrate donated sulfur and a tryptophan it would be expected that mostly solvent accessible residues would be quenched. This is not the case with rhodanese.

The partial primary sequence of rhodanese (10) shows that the tryptophan residues are distributed fairly uniformly along

Table I

Fluorescence Parameters of Sulfur Free (E) and Sulfur Substituted (ES) Rhodanese

	Q_{ES} (a)	Q_E/Q_{ES}	$\lambda_{max\ ES}$ (nm) (b)	$\Delta\lambda_{max}$ (nm) (c)
Direct Spectrum in 2 M NaCl Figure 1, Curve I	.23	1.3	333	0 ± 1
Direct Spectrum in 0.016 M Tris (d)	.22	1.3	332	0 ± 1
Unquenched residue spectrum (Figure 2, Curves I & II)	.15	1.3	324	0 ± 1
Quenched residue spectrum (Figure 2, Curves III & IV)	.08	1.3	340	0 ± 1

(a) Quantum yields calculated based on the total tryptophan absorption

(b) Results reproducible to 1 nm

(c) $\Delta\lambda_{max} = \lambda_{max\ E} - \lambda_{max\ ES}$

(d) Data not shown

the chain of 317 amino acids with the active site sulfhydryl group being more than 50 residues from the closest tryptophan. It therefore appears unlikely that even in the tertiary conformation of rhodanese a localized contact mechanism could give rise to the general quenching seen here.

A non-radiative energy transfer mechanism, on the other hand, depends mainly on the distance between the donor and acceptor and the mutual orientation of their transition dipoles. Quenching by this mechanism would be independent of the solvent accessibility of the tryptophan residues. As Figure 2 and Table I demonstrate, this appears to be the case with rhodanese.

Although it is possible that part of the observed quenching may result from the conformational change known to occur on sulfur binding (11,12), it appears that of the two commonly

proposed quenching mechanisms, non-radiative energy transfer is far more likely. Therefore, even if direct contact contributes to the observed fluorescence behavior, the data here supports the proposal of a persulfide intermediate in rhodanese catalysis (3,13).

Acknowledgment

Acknowledgment is made to the donors of the Petroleum Research Fund, administered by the American Chemical Society, for support of this research.

We would like to thank Dr. R. F. Chen for supplying a corrected emission spectrum of bovine serum albumin.

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