# ON THE NATURE OF TYROSINE PHOSPHORESCENCE FROM PROTEINS[1]

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Early theories of energy transfer among aromatic amino acids proposed that the emission from tryptophan-containing proteins should be characteristic solely of that residue (see a review by Stryer, 1960). More recent studies, however, led Teale (1960) to conclude that the nature of the variation with excitation wavelengths in the emission yields of tryptophan fluorescence from proteins indicates that the efficiency of interresidue energy transfer from tyrosine to tryptophan is very small for wavelengths between 280 and 310 nm. Further, there have been recent reports of extensive tyrosine phosphorescence (Longworth, 1962; Augenstein and Nag-Chaudhuri, 1964)--and perhaps tyrosine fluorescence (Teale, 1961; Weber, 1961)--in a number of tryptophan-containing proteins. We anticipated that this reflects environment-sensitive perturbations of specific tyrosine residues in the various proteins, since some tyrosine residues have been shown previously to be perturbed electronically in RNase, whereas others apparently are not (Nag-Chaudhuri and Augenstein, 1964).

To analyze this possibility it is necessary to determine how much tyrosine phosphorescence is emitted from various proteins. An exact determination is difficult since there is considerable spectral overlap of the phosphorescence emission characteristic of tyrosine and tryptophan. In this report we analyze some factors which modify tyrosine phosphorescence and emphasize that that from proteins is not necessarily identical with that from tyrosine alone in

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solution. Utilizing this knowledge we have developed a method for resolving the phosphorescence spectra and have estimated the relative amounts of tyrosine and tryptophan phosphorescence from a group of proteins.

For our spectroscopic studies we used either: (a) solutions containing 0.1 mg/ml of tyrosine, tryptophan, polytyrosine, or one of 15 proteins dissolved in a 2:1 mixture of purified glycerol and $H_2O$; or (b) powders prepared by lyophilizing solutions of tyrosine, tryptophan or amino-acid mixtures (see below) dissolved in $H_2O$ alone. The phosphorescence emission spectra of these samples in 2 mm. cylindrical capillaries were recorded at 77°K with an Aminco spectrophosphorimeter (Drobnik and Augenstein, 1966) and were corrected for instrumental response.

In Fig. 1A and 1B are plots of the phosphorescence emission spectra for tyrosine and tryptophan in various environments. Fig. 1C contains analogous plots for RNase, lipoxidase, α-chymotrypsin and a resolution of the lipoxidase spectrum into "tyrosine" and "tryptophan components". Clearly the emission from RNase--a protein, like insulin, which contains no tryptophan--is much broader than that of tyrosine alone in solution and its maximum is at 415 nm rather than 390 nm. However, the shape of the RNase spectrum, the phosphorescence lifetime and the position of the maximum are not characteristic of that from powders of tyrosine crystals (J. Nag-Chaudhuri and L. Augenstein, 1964) nor of powders formed by lyophilizing tyrosine alone. Further, the RNase emission is very similar to that from either the lyophilized tyrosine-glycine mixture or from polytyrosine in solution. From these results we conclude (a) that lyophilization of tyrosine alone in solution leads to the formation of at least some tyrosine crystals, whereas this is not the case for lyophilization of a tyrosine-glycine mixture (an analogous situation appears to occur with tryptophan) and (b) that the emitting tyrosine residues in RNase are perturbed as a result of interactions with neighboring polypeptide groups. Accordingly, in resolving the phosphorescence spectra from tryptophan-containing proteins into tyrosine and tryptophan components we take the spectral shape of the

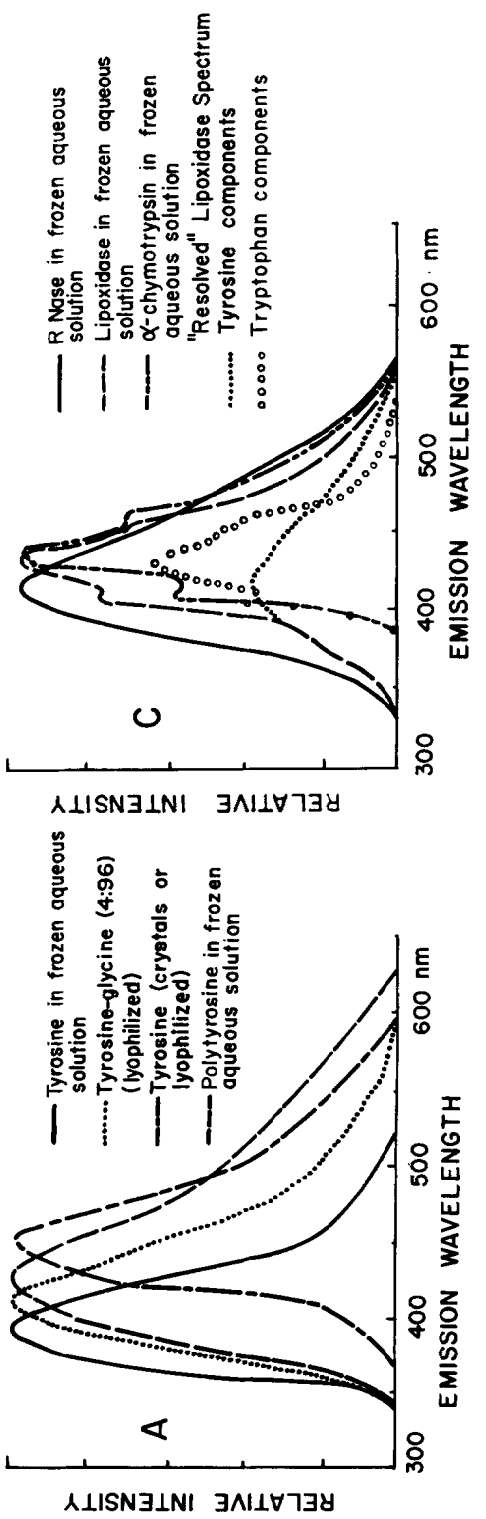


Figure 1. The phosphorescence emission spectra from (A) tyrosine and (B) tryptophan in various environments and (C) from three proteins. The spectrum from lipoxidase has been resolved into tyrosine and tryptophan components (see text). The emission from an amino acid mixture having the same composition as α-chymotrypsin has a spectrum identical in shape to that from tryptophan -- that from a "lipoxidase amino acid mixture" has a shape similar to the phosphorescence from a lyophilized tyrosine-glycine mixture or from RNase.

emission from tyrosine residues in proteins to be the same as the emission from RNase.

The phosphorescence from α-chymotrypsin is essentially the same as that from tryptophan excited either in the frozen matrix or in a lyophilized glycine-tryptophan powder. Thus, there must be almost no tyrosine phosphorescence from this protein and further the emitting tryptophan residues must be essentially unperturbed both in this protein and also in all the other environments tested with the exception of crystals. Clearly tryptophan does not phosphoresce at wavelengths below 390 nm (Fig. 1B) and the decay of phosphorescence emitted from proteins at shorter wavelengths has a lifetime characteristic of tyrosine (Augenstein and Nag-Chaudhuri, 1964). Thus, by assuming that the emission spectrum for the tyrosine residues in all proteins is the same as that from RNase, it is possible to normalize the spectrum of the tyrosine components in a given protein to the intensity observed at 390 nm. Subtracting the normalized tyrosine values from the total quanta emitted at a given wavelength provides an estimate of the tryptophan phosphorescence components. As can be seen in Fig. 1C, resolving the lipoxidase data in this fashion gives estimated "tryptophan components" which are almost identical in shape to the normal tryptophan phosphorescence emission spectra.

Utilizing this methodology to resolve the phosphorescence spectra obtained from the various proteins studied gave the values listed in Table I. There it can be seen that from most of the proteins a significant fraction of the energy in excited tyrosine residues is not transferred to tryptophan residues: this is particularly evident for excitation by 240-nm light in which the fraction of photons absorbed by phenylalanine and tyrosine residues is greater than that when excitation is at 280 nm. However, it is not possible to correlate the fractions of tyrosine phosphorescence with the fractions of either tyrosine or tryptophan residues in the various proteins (for which amino-acid compositions are available).

Table I: Percentage of phosphorescence from tyrosine residues of various proteins (±15%). Duplicate measurements are given in most cases.

| Protein | Excitation Wavelength 240 nm | 280 nm |
|---|---|---|
| alcohol dehydrogenase[c] | 98,80 | 76,51 |
| α-chymotrypsin[b] | 0 | 0 |
| carboxypeptidase[a] | 51,44 | 46,45 |
| collagenase[c] | 75,65 | 73,52 |
| fumerase[a] | 107,98 | 85,94 |
| glycerol phosphate dehydrogenase[c] | 110,90 | 77,55 |
| hyaluronidase[b] | 68,69 | 49,52 |
| insulin[a] | 103,91 | 106,97 |
| lipoxidase[b] | 68 | 60 |
| lysozyme[b] | 58 | 14 |
| malic dehydrogenase[c] | 91,107 | 78,80 |
| ovomucoid[b] | 97,94 | 59,77 |
| RNase[b] | 100 | 100 |
| trypsin[b] | 13 | 8 |
| trypsin inhibitor[b] | 45 | 28 |

Sources: [a]Cal Biochem; [b]Worthington Biochemical Corp; [c]General Biochemicals

We are investigating further the preliminary results with two lyophilized amino-acid mixtures which suggest that the relative probabilities of tyrosine intersystem crossing and singlet-singlet, tyrosine-to-tryptophan transfer may depend critically upon the average tyrosine-tryptophan separation: i.e., emission from the "α-chymotrypsin amino-acid mixture (2.5% tyrosine and 5% tryptophan)" is almost exclucively characteristic of tryptophan whereas that from the "lipoxidase mixture" (6% tyrosine and 0.4% tryptophan) has a large fraction typical of tyrosine. Eventhough lysozyme has 11% tryptophan, the relatively large tyrosine phosphorescence observed from this protein may reflect spatial "isolation" of one tyrosine residue, since current crystallographic data (Blake et al., 1965) indicate that residue no. 53 may be 15A or more from the nearest tryptophan. Two observations make it unlikely, however, that the extensive "tyrosine phosphorescence" in all proteins is simply the result of large spatial separation of some tyrosine and tryptophan residues so as to prevent energy transfer:

a) there is no uniform relationship between the fractions of tyrosine phosphorescence arising from excitation at 240 and 280 nm and b) we do not observe the extensive fluorescence which would be expected from isolated emitting tyrosine residues. That the phosphorescence excited (either at 240 and 280 nm) from both α-chymotrypsin and its amino-acid mixture are typical of that from tryptophan alone, implies that either there is appreciable quenching of either

or both excited tyrosines and/or tryptophans or else in contradiction to Teale's (1960) conclusion there is appreciable energy transfer from at least some excited tyrosines to tryptophans. The present results indicate that either of these possibilities must vary from protein to protein and, presumably, depend critically upon specific perturbations arising from the local environments of certain aromatic residues. Hopefully the studies with amino-acid mixtures now underway may provide an insight into conditions which could enhance singlet-triplet intersystem crossing in tyrosine residues.

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