THE CHARACTERISATION OF PROTEINS BY SYNCHRONOUS AND DERIVATIVE LUMINESCENCE SPECTROSCOPY

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Studies of protein photochemistry and structure by high resolution luminescence spectroscopy have been limited by poor detection of tyrosine (Tyr) in the presence of tryptophan (Trp) residues. The excitation spectra and the broad fluorescence spectra of these two amino acids overlap strongly, especially since a blue shift in Trp emission is often observed in proteins. Spectra with narrower bandwidth and greater specificity may, however, be produced by synchronous scanning fluorimetry (Lloyd 1971), where the excitation and emission monochromators separated by a fixed wavelength interval ($\Delta\lambda$) are scanned simultaneously. The interval $\Delta\lambda$ represents a unique flexible element for spectral resolution (Miller 1979). In the present study, this new approach in analytical biochemistry has been combined with second derivative fluorimetry to generate typical profiles for characterisation of proteins by their aromatic amino acid residues.

Fully corrected synchronous scanning of binary mixtures of Tyr and Trp in 0.1M Tris HCl buffer (pH 7.4) (solvent A) generates a single sharp Tyr peak at the emission wavelength (λf) 294nm for $\Delta\lambda$ at 10nm (spectral bandwidth = 2.0nm for both monochromators). With $\Delta\lambda$ at 70nm, two overlapping peaks are observed which correspond to Tyr (λf = 300nm) and Trp (λf = 350nm). Calibration graphs for binary combinations of Tyr ($\Delta\lambda$ = 10nm) and Trp ($\Delta\lambda$ = 70nm) at a total concentration of 10μM were linear and passed through the origin, the correlation coefficients being 0.9998 and 0.9990 respectively (n = 6). The luminescence intensity of each component was mutually independent. For the ternary system with phenylalanine (Phe), synchronous scanning with $\Delta\lambda$ at 3nm permits resolution of Phe (λf = 268nm) overlapped by Tyr (λf = 287nm). However, the low quantum yield of Phe limits its detection to 10 μM in this system. Linear functions of luminescence intensity observed for Phe (10 to 50 μM) and Tyr (0 to 5 μM) in ternary mixtures with 5 μM Trp were mutually independent.

With $\Delta\lambda$ at 3nm for Tyr and 70nm for Trp, the synchronous spectra of dilute well-defined native proteins in solvent A were compared with those for proteins denatured by 5.4M guanidine HCl (solvent B), and the characteristic λf values and molar ratios of Tyr:Trp recorded. Phe residues were not detected in these proteins.

	Concanavalin A		Human Transferrin		Ovalbumin		Bovine Serum Albumin	
Solvent	A	B	A	B	A	B	A	B
λf nm (Tyr)	295	290	294	288	289	288	291	288
λf nm (Trp)	340	350	339	352	342	345	345	345
molar ratio	1.2	1.4	2.0	2.5	1.1	2.0	0.8	3.8

The Trp residues are red-shifted in denatured proteins, for which Tyr:Trp ratios agree well with structural data, except for bovine serum albumin, where multiple sulphide bonds may hinder complete denaturation. Transformation of the synchronous spectrum ($\Delta\lambda$ = 70nm) to its second derivative using an analog microprocessor gives excellent and more rapid resolution of the Tyr and Trp peaks in a single spectrum, with significant reduction of interference by the fluorescent matrix. These techniques have also been applied to studies on human serum albumin, lysozyme and the tyrosine-rich proteins ribonuclease A and bovine insulin. Synchronous scanning and its second derivative analog offer a rapid and sensitive luminescence probe for studies on drug-protein interactions.

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