Biochemical Fluorescence

CONCEPTS

edited by
Raymond F. Chen
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Volume 2

Biochemical Fluorescence: Concepts

VOLUME 2

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生化萤光：概念　第2卷

本书介绍萤光方法在生物化学研究中的各种用途。这一卷是介绍萤光在研究蛋白质、多肽激素、底物类似物、核黄素、酶、药物、多核苷酸、膜、抗体等方面的应用。可供生物化学、分子生物学有关的科研工作者参考。

目次：⑧蛋白质发射光谱的异质性，⑨蛋白质和模型多肽中的酪氨酸萤光，⑩进行构象变化中的蛋白质发射谱带形状的变化，⑪内禀蛋白萤光的微扰，⑫多肽激素的结构，⑬金属阳离子对蛋白内禀萤光的影响，⑭合成萤光底物类似物，⑮游离及结合核黄素萤光，⑯谷氨酸脱氢酶结构转换中的内禀及外来萤光，⑰药物-蛋白相互作用的萤光，⑱多核苷酸的阳离子萤光探测，⑲膜结构和功能研究中的萤光探测，⑳生物膜能化态的萤光探测，㉑免疫球蛋白异质性的萤光测定，㉒抗体构象的共振能量转移研究，㉓抗体活性部位的萤光探测。

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PREFACE

There was a fleeting moment during the years when fluorescence spectroscopy was maturing as a biochemical method when it seemed that fluorescence might be too nonspecific to be ultimately useful. For example, the intrinsic fluorescence of most proteins seemed quite similar, and the spectra all looked alike when the proteins were denatured. As for extrinsic fluorescence due to dyes adsorbed onto macromolecules, this also seemed of limited value: there were few useful dyes, and compounds such as ANS seemed to exhibit enhanced fluorescence only with serum albumin. Perhaps the most important factor which reinforced faith in fluorescence methods was the gradual accumulation of data showing that fluorescence parameters did, in fact, vary according to the macromolecule under study. In other words, when one looked closely enough, proteins were not all alike. In addition, a variety of covalent and adsorbed fluorescent probes were shown to yield significant data with a wide range of macromolecules. By examining energy transfer and other fluorescence parameters with modern instrumentation, investigators found that emission spectroscopy provided information not available through other methods.

Later, it seemed as if the pendulum would swing too far to the other direction; it was so easy to get fluorescence data that everyone seemed to be working at a spectrofluorometer. ANS was looked on as a universal probe which could be applied to any system, resulting in a new set of publications. Every macromolecule was said to have its own emission characteristics, and frequently that was the only justification for the publication of spectral data. Fortunately, the trend now seems to be to demand that the observations be interpretable. This aim is achievable by combining fluorescence

with other physical methods, using more specific fluorescent probes, and by careful preparation of well-defined systems, such as those for energy transfer studies.

Just as nuclear magnetic resonance, a brainchild of physics, has found its greatest utility in chemistry, so the rich flowering of biochemical fluorescence could not have been foreseen by the physicists who first studied photoluminescence. The chapters in this volume reflect some of the newer areas of application: membrane biophysics, antibody structure, new fluorescent probes, perturbation probes, refinements in the study of intrinsic protein luminescence, and so forth. While Volume I dealt mainly with the theoretical basis of fluorescence spectroscopy, it is clear from both volumes that experimental observations frequently demand explanation and thus give rise to the development of theory.

Each contributor to these volumes has been asked to indicate the future directions which will be taken in the areas discussed in each chapter. These prognostications should be of interest to students and experienced investigators alike. In fact, since receipt of the completed chapter manuscripts, we have noted that many of the predictions by the authors have already begun to be fulfilled. For this reason, the editors are hopeful that CONCEPTS will provide a framework from which developments in biochemical fluorescence can be understood.

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CONTENTS OF VOLUME 1

CONTENTS

Chapter 8

HETEROGENEITY IN PROTEIN EMISSION SPECTRA

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INTRODUCTION

The usefulness of emission spectroscopy in probing the structure and dynamics of proteins can be enhanced with the recognition of the heterogeneous nature of protein emission spectra. In the present article, I would like to draw attention to the evidence which exists for this heterogeneity and to the type of information it can be expected to provide. No attempt is made to comprehensively review all of the early work in this area. Much of this information is contained in a recent review by Longworth [1] to which the reader is referred, and earlier discussions can be found in articles by Weber and Teale [2] and in the book by Konev [3]. The purpose here is to try to present a consolidated picture in the light of more recent developments in the field. In particular, emphasis is placed on the utility of phosphorescence measurements not only in resolving the presence of components in phosphorescence spectra, but in addition in providing evidence for the variability in absorption and fluorescence which obtains for different chromophores within the same protein molecule. I have purposely concentrated on tryptophan emission in proteins in that it has received the most attention experimentally, and a clearer understanding of the factors influencing the emission properties has emerged for tryptophan than for tyrosine.

II. GENERAL CONSIDERATIONS

Heterogeneity will be observed within an emission spectrum when two or more independently emitting species possessing distinct emission properties contribute to the overall emission. In order for this to occur, two requirements must be met. First there must be subclasses of molecules within the sample with inherently different emission spectra. The subclasses can be chemically distinct chromophores such as tyrosine and tryptophan, or could represent molecules of the same species which differ either with respect to their ionization state or to the perturbations they experience as a consequence of their location in particular local environments. Second, excitation and emission for each of the subclasses of emitting molecules

in the sample must occur independently. Electronic coupling between molecules with distinct emission properties leads to localization of the excitation on the chromophore with the lower excitation energy, and emission will be observed only from it. This situation prevails with DNA. In the native state, even relatively short-range triplet delocalization [4,5] leads to trapping of the excitation by thymine, and hence only thymine phosphorescence is observed [6], while adenine and guanine phosphorescence appear with denatured DNA [7] where the transfer is less efficient [5].

The natural structure of proteins is conducive to the manifestation of heterogeneity within their emission spectra. Significant contributions to the emission spectra of proteins come from tyrosine and tryptophan which display distinct fluorescence spectra and even more clearly discernible phosphorescence spectra. It is well known that tryptophan dominates the fluorescence from proteins possessing several tryptophans, but tyrosine fluorescence is also observed particularly in those proteins which contain tyrosine but no tryptophan [8]. More significant from the point of view of the present discussion are the early observations of Teale [8] that the tryptophan fluorescence maximum varies from protein to protein, and those of Konev [3] which indicated a similar variation in the tryptophan phosphorescence maxima of several proteins. It follows that proteins satisfy the first requirement in that not only do they possess tyrosine and tryptophan both of which are capable of emitting, but the tryptophan spectra themselves display variations from protein to protein. If the overall tryptophan spectra can vary from one protein to another, it is natural to inquire whether distinct emission might not be observed from different tryptophans within the same protein molecule.

A. Singlet Interactions Between Aromatic Residues

The ability to observe independent emission from distinct intrinsic chromophores within a protein depends on the degree of coupling, or more precisely the lack of coupling, between them.