Methods in Enzymology

Volume 61

Enzyme Structure

Part H

EDITED BY

C. H. W. Hirs

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Preface

This is the third of three volumes of "Enzyme Structure" devoted to updating the treatment of physical methods (parts F and G appeared last year). Although coverage of the various techniques is not exhaustive, it is hoped that the intent of presenting a broad coverage of currently available methods has been reasonably fulfilled. The organization of the material is the same as in the previous volumes.

As in the past, these volumes present not only techniques that are currently widely available but some which are only beginning to make an impact and some for which no commercial standard equipment is as yet available. In the latter cases, an attempt has been made to guide the reader in assembling his own equipment from individual components and to help him find the necessary information in the research literature.

In the coverage of physical techniques, we have departed somewhat in scope from the traditional format of the series. Since, at the termination of an experiment, physical techniques frequently require much more interpretation than do organic ones, we consider that brief sections on the theoretical principles involved are highly desirable as are sections on theoretical and mathematical approaches to data evaluation and on assumptions and, consequently, limitations in the applications of the various methods.

We wish to acknowledge with pleasure and gratitude the generous cooperation of the contributors to this volume. Their suggestions during its planning and preparation have been particularly valuable. Academic Press has provided inestimable help in the assembly of this volume. We thank them for their many courtesies.

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METHODS IN ENZYMOLOGY

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Section I

Molecular Weight Determinations and Related Procedures

[1] Pulsed Laser Interferometry in the Ultracentrifuge

By DAVID A. YPHANTIS

The principal advantages of continuous laser light sources for Rayleigh interferometry in the ultracentrifuge lie in the increased convenience and lower operating costs associated with relatively long-lived lasers that require no water cooling and in the greatly increased monochromaticity and coherence length of the laser light compared to the usual high pressure mercury illumination. These advantages are discussed in detail in Volume 48 of this series.[1] The additional benefits of pulsed laser interferometry stem largely from the precise control of illumination timing and the high light intensities that are readily available.

Two experimental arrangements have been described for utilizing pulsed laser light in the ultracentrifuge: One arrangement employs a pulsed argon ion laser of moderately high intensity[2] while the other modulates a low power helium–neon gas laser either externally with a Pockel cell[3] or by means of internal switching.[1] Both arrangements make possible the illumination of (tangentially) selected regions of the rotor and cells. The Pockel cell modulation system makes possible finer control of the region to be illuminated because of its shorter pulse length, down to 0.8 μsec compared to the ~6 μsec for the unmodified pulsed argon ion laser. On the other hand, the argon ion laser emits significantly higher light intensity than the He–Ne lasers used and, in addition, can provide a number of laser lines in the green and blue region of the spectrum. The pulsed argon ion laser system used in this laboratory for several years is described here.

Apparatus

The argon ion laser used has been manufactured intitially by TRW as the 83-A (and 83-AR, when a manual refill system has been included) and later by Quantrad as the Model 83-AR (83-AB with a modified refill system) and 93 laser. The maximum repetition rate of the ~6 μsec long pulses is limited by the line frequency to 60 pulses/sec. The lasers used provide 0.8 W (peak power) of light in the TEM 00 Mode at 5145 A. Nearly as much power is available at 4880 A and considerably less at

[1] R. C. Williams, Jr., Vol. 48, p. 185.
[2] C. H. Paul and D. A. Yphantis, *Anal. Biochem.* **48**, 588 (1972).
[3] J. A. Lewis and J. W. Lyttleton, *Anal. Biochem.* **56**, 52 (1973).

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