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Simultaneous measurements of fast optical and proton current kinetics in the bacteriorhodopsin photocycle using an enhanced spectrophotometer

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Abstract

A one-of-a-kind high speed optical multichannel spectrometer was designed and built at NIH and described in this journal in 1997 [J.W. Cole, R.W. Hendler, P.D. Smith, H.A. Fredrickson, T.J. Pohida, W.S. Friauf. A high speed optical multichannel analyzer. J Biochem Biophys Methods 1997;35:16–174.]. The most unique aspect of this instrument was the ability to follow an entire time course from a single activation using a single sample. The instrument has been used to study rapid kinetic processes in the photon-driven bacteriorhodopsin photocycle and electron transport from cytochrome *c* to cytochrome aa_3 and from cytochrome aa_3 to oxygen. The present paper describes a second generation instrument with a number of important enhancements which significantly improve its capabilities for multichannel kinetic studies. An example application is presented in which the kinetics of photon-induced proton flow across the biological membrane is measured simultaneously with the individual steps of the photocycle determined optically. Matching the time constants for the two processes indicates which molecular transformations are associated with major proton movements.
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Keywords: High speed spectrophotometer; Rapid kinetics; Bacteriorhodopsin photocycle; Proton pump; Energy transduction

1. Introduction

Prior to the introduction of analytical procedures capable of deconvoluting whole spectra that developed in a third dimension such as pH or solution potential [1], analyses were based on experimental data collected at either a single characteristic wavelength or the characteristic wavelength and a reference wavelength such as an isosbestic point. For such thermodynamic studies, ordinary spectrometers can be used. To apply the same analytical procedures to kinetic studies required a high speed optical multichannel spectrometer. For fast kinetic processes that could be quickly initiated by a laser pulse, existing spectrometers capable of rapid acquisition of whole spectra were based on using a separate actinic and monitoring light pulse for each time point. To use the new multichannel analyses [1], a new kind of spectrometer that could follow the entire time course after a single actinic pulse would be highly desirable. Such a spectrometer was designed and built at the National Institutes of Health [2]. Hendler and collaborators used this spectrometer in an extensive series of kinetic studies on various aspects of the bacteriorhodopsin photocycle [3–10] and on electron transfer to and from mammalian cytochrome oxidase [11,12]. During these studies with the original instrument over a 14 year period, it became obvious that a newer version of the instrument with added capabilities would enable a more thorough examination of kinetic processes. Specifically, the resolution was increased from 12-bits to 16-bits, the maximum sampling rate was increased from 100 kHz to 200 kHz, independent programmable gain and offset for each channel were added, logarithmic time scheduling was added as an

Abbreviations: DAQ, data acquisition system; PCB, printed circuit board; ADC (A/D), analog-to-digital conversion; DSP, digital signal processor; CIO-CTR10, PC plug-in timer board; CLD, configurable logic device; PM, purple membrane; BR, ground state of bacteriorhodopsin; SVD-lsq, singular value decomposition-based least squares; OD, optical density.

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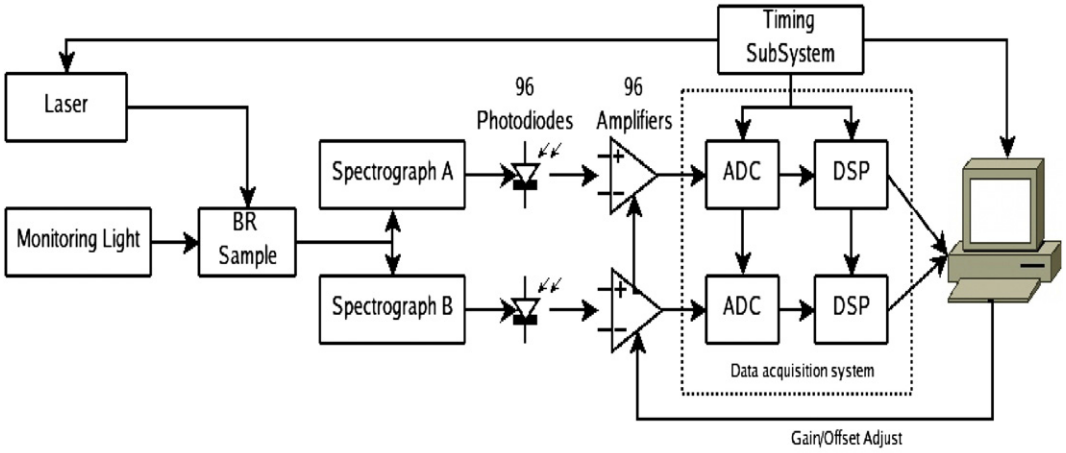

Fig. 1. Overall spectrometer system diagram.

option, and system stability and reliability were enhanced. In addition, an extra channel was enabled so that the kinetics of proton electric current flow could be measured simultaneously with the optical kinetics of the system. Bacteriorhodopsin, like cytochrome oxidase, is an energy-driven proton pump which converts an input energy into an electrochemical gradient across the membrane, from which ATP is synthesized. In the case of cytochrome oxidase, the energy source is from respiratory electron transport, whereas with bacteriorhodopsin the driving force is from absorbed photons. The photon energy is used to electrogenically pump protons across the membrane to build the electrochemical gradient. The ability to match electrogenicity to specific transitions in the photocycle, followed optically, helps identify which steps on the photocycle are most involved in the energy transduction process. Experiments to accomplish this goal using the instrument described here have been performed (paper in preparation). The programmable offset feature is important because certain photodiodes produce negative dark currents which register as zeros in the A/D converter, thus requiring intricate calibration procedures to obtain the true dark spectrum. While the original instrument was built completely in-house with common prototyping methods, the upgraded version uses commercially available components for the data acquisition system and custom printed circuit boards for the front-end amplifiers and timing system.

2. Materials and methods

2.1. Instrumentation

2.1.1. Spectrometer

The electronics (Fig. 1) consist of the analog front-end, the data acquisition system (DAQ), the timing subsystem, and the PC. The analog front-end amplifies the photodiode output (providing adjustable gain and offset) that is then fed into the DAQ system which performs the analog-to-digital conversion and sample processing (only a subset of all samples is retained). The digitized data are transferred to a PC where they are displayed and saved to disk. The PC is also used to configure the system. The timing subsystem (consisting of a counter-timer board and some custom electronics) provides sampling clocks, triggers, and laser control signals. A graphical user interface to control the system was written using the Microsoft Visual Basic programming language.

The custom-built analog front-end (Fig. 2) consists of the photodiode arrays and amplifiers. Two photodiode arrays (Model #S4112-46, Hamamatsu Photonics, Hamamatsu City, Japan) are used, each mounted on separate printed circuit board (PCB). Each board is mounted inside a small aluminum box with a hole cut such that the face of the photodiode array extends slightly beyond the box outer surface. The boxes are

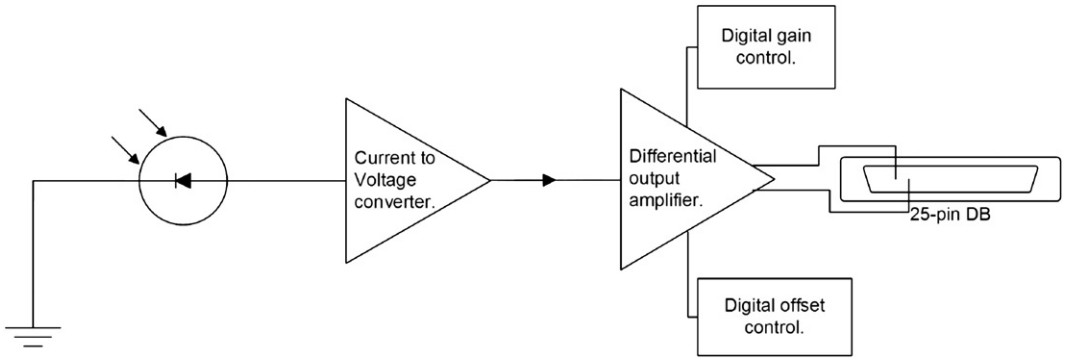

Fig. 2. Diagram of a single analog channel.