Electronic Equipment for the Measurement of Weak Biological Luminescence

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Equipment for measuring intensity and spectral composition, such as the microprocessor-controlled TD-7 Chemiluminescence Counter /Showa Denko, Japan/ /IMABE et al., 1982/ is at present available on the market. These programmed temperature devices are equipped with a set of limiting or interference filters and a set of measuring cells to be used with samples of different consistencies. Such devices are, however, expensive, which means that many laboratories are unable to afford them.

A measuring device has thus been invented which is relatively easy to assemble from ready-made elements, and which is versatile for use in weak biological luminescence assays. It makes it possible to measure the kinetics, spectral distribution and temperature dependence of weak luminescence in solutions and biological systems.

Electromagnetic radiation emission /luminescence/ within the 170-800 nm range is associated with radiant transfer from the lowest electron-activated states to the basic states of the molecules.

The activation energy may be transferred either chemically /1/ or physically /e.g. 2/:

$$PS \xrightarrow{\text{reaction}} P^{x} \longrightarrow P + h\nu \qquad /1/$$

where PS is the substrate, P^{x} is the activated product, and $h\nu$ is the light quantum.

$$PS + h\nu \longrightarrow P^{x} \longrightarrow P + h\nu' \qquad /2/$$

The primary $(=C=O)^{x}$ products, generated in the activated states, include carbonyl compounds, molecular oxygen and condensed aromatic compounds.

Weak luminescence /chemiluminescence/, resulting from chemiactivation, accompanies such reactions as oxidation, the synchronous disruption of bonds, and radical recombination. Chemiluminescence has been employed for analytical purposes.

Chemiluminescence /CL/ emitter identification involves comparing a CL spectrum with that of the fluorescence /Fl/ or phosphorescence of the same molecule within the same spectral range.

A live cell contains numerous compounds capable of fluorescing, e.g. flavines, chlorophylls, nitrogen bases, quinones, tryptophan rests in proteins, etc. All these compounds are activation energy acceptors. The probability of energy transfer from the primary activation products P^X to acceptors is thus high.

Tissues, cells, and their homogenates, as well as organelles, emit spontaneous luminescence. The integral intensity of the weak biological luminescence of living organisms depends on their metabolic activity. A statistically significant correlation was found between the intensity /I/ or light sum

$$(\Sigma I = \int_{t=0}^{t=\infty} I\ /t/\ dt)$$

and the seed germination energy or mitochondrial suspension activity /MILCZAREK, 1983; MILCZAREK et al., 1973, 1974/.

The "radiation response" of a living organism to changes in temperature T, as expressed by the function I = f/T/, yields information on the resistance of the organism to the rate and range of changes in T. By recording luminescence intensity as a function of T, changing at a constant rate dT/dt and within a sufficient temperature range during a test run, the so-called low- and high-temperature emission peaks and hysteresis curves are obtained /Fig. 1/.

The area within the curve may represent a homeostasis disturbance in an open system such as a living organism.

Principles of design and function

The electronic apparatus designed to measure the intensity and spectral composition of weak biological luminescence consists of a measuring chamber and a supply/record system /Fig. 2/.

The measuring chamber is composed of a lightproof camera /12/, a photomultiplier to be used as a detector /3/, an automatic limiting filter changing device /2/, a measuring cell /1/ and temperature control units /10, 11/.

The supply/record system consists of the following blocks: high voltage supply unit /5/, single-electron pulse amplifier /4/, signal generator /6/, computer /7/, low voltage supply unit /8/ and thermo-control system supply unit /9/.

The weak emission from biological systems requires the use of photomultipliers for the minimal dark currents and a high spectral photocatode sensitivity within as wide a spectral range as possible /170-900 nm/. These requirements are met by the 9658 EMI /England/ and T-818 Hamamatsu /Japan/ photomultipliers; the Zeiss M12 FVC51 /GDR/ instrument is less effective. The light pulses accompanying the reaction or phenomena studied are converted within the detection system /3/ into electric pulses of varying amplitudes /Fig. 3/. These pulses are amplified by a wide band amplifier /4/ and passed to the generating system /6/ to be processed into digital or analogue records in the computer /7/. Simultaneously, the computer programmes and controls the test run.

The system described can be relatively easily altered or modified, according to the user's needs.

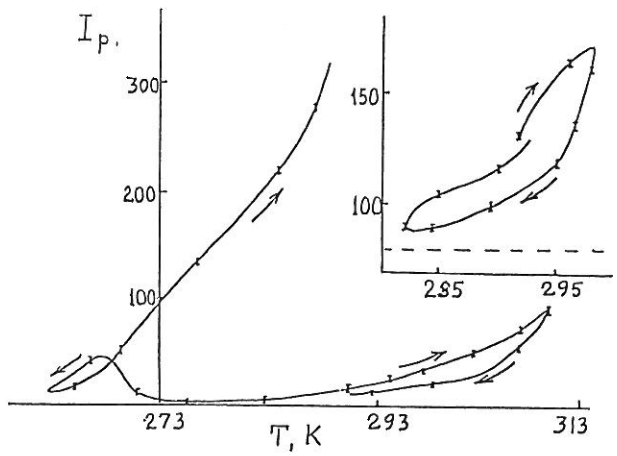

Fig. 1

"Temperature hysteresis" curve of spontaneous weak luminescence of barley germs at two different temperature cycles; constant rate of temperature change $dT/dt = 0.5 \cdot K\ min^{-1}$. /Arrows denote the direction of changes in T/

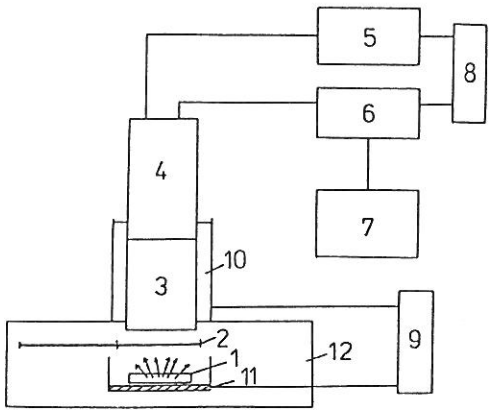

Fig. 2

Block diagram of electronic measuring apparatus designed to study weak biological luminescence and chemiluminescence. 1. measuring cell; 2. automatic limiting filter changing device; 3. photomultiplier; 4. single-electron pulse amplifier; 5. high voltage supply unit; 6. signal generator; 7. computer; 8. low voltage supply unit; 9. thermocontrol system supply unit; 10, 11. temperature control units; 12. light-proof camera

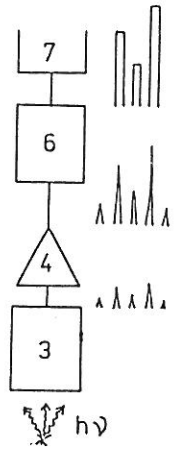

Fig. 3

Functional diagram of photomultiplier /3/, amplifier /4/ and signal generating system /6/

References

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