CHEMILUMINESCENCE OF ATP SOLUTION

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Radiation in the visible region of the spectrum (400–500 nm) from a 0.02 M aqueous solution of ATP at 37°C was recorded with a photoelectronic multiplier. The radiation was shown to depend on the pH of the solution. It is absent in an acid medium; the intensity of chemiluminescence at pH 7.0–7.5 is about 200 ϕ/sec/ml, and with an increase in pH to 10 it rises sharply – to 1,500 ϕ/sec/ml. The quantum yields of radiation were 10^{-16}–10^{-17} in a neutral and 10^{-15}–10^{-16} in an alkaline medium.

KEY WORDS: ATP; chemiluminescence.

ATP is now regarded as the principal accumulator and donor of energy for cell processes. According to Szent-Gyorgyi [3, 5], one possible mechanism of function of this molecule is connected with the weak transfer of the charge within the ATP molecule itself. Under these circumstances, excited states of the molecule may arise as rare events and one manifestation of them could be the production of photic quanta.

The writer has shown [1] that radiation with a wavelength of over 350 nm, with an intensity of about 200 ϕ/sec/ml, and with a quantum yield of 10^{-16}–10^{-17} can be reliably recorded by means of a photoelectronic multiplier (PEM) from an aqueous solution of ATP at pH 7.0–7.5 and at 37°C. It is interesting to note that the addition of ATPase (actomyosin) to such a solution causes the appearance of an ultraviolet component, with an intensity of 200–250 ϕ/sec/ml, in addition to the visible radiation. The presence of the weak ultraviolet (mitogenetic) radiation during interaction between ATP and ATPase has also been demonstrated by the use of a biological detector [1]. The appearance of mitogenetic radiation during interaction between substrate and enzyme is a characteristic feature of many enzymic reactions [2].

The object of this investigation was to study the relationship between the chemiluminescence of an ATP solution and its pH value.

EXPERIMENTAL METHOD

The technique and the scheme of the apparatus were fully described previously [1, 4]. The PEM used was a type FÉU-18A instrument with quantum yield of 2% for 250 nm and 5% for 450 nm. The PEM operated under photon counting conditions. Glass and quartz filters were used. The background thermal emission was 2–6 pulses/10 sec. The "glass–background" pulse difference corresponded to visible radiation with a wavelength of over 350 nm (the short-wave limit of the transmission band of the glass filter), whereas the "quartz–glass" difference corresponded to ultraviolet radiation with a wavelength of below 350 nm. The solutions were placed in a constant-temperature quartz cell with a capacity of 10 ml. The pH was altered by adding either 2 N HCl or 5 N KOH to the ATP solution. The measurements taken with 0.02 M ATP solution at different pH values are given in Table 1. In an acid medium at pH 0.2 and 3.4, when the intensity of nonenzymic hydrolysis is far higher, no radiation was recorded. Weak radiation with an intensity of about 200 ϕ/sec/ml appeared within the range pH 7.0–7.5. With an increase in pH the intensity of the radiation rose sharply, reaching 1,500 ϕ/sec/ml at pH about 10, beyond which it weakened slightly. The radiation of all solutions tested lay in the visible region of the spectrum with a wavelength of over 350 nm, for in every case the "quartz–glass" pulse difference was within the scatter of the measurements.

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TABLE 1. Radiation of a 0.02 M ATP Solution at 37°C in the Visible Region of the Spectrum during the First 10 min of Measurement (M ± m)

Solution tested	pH	Mean "glass – background" pulse difference in 10 sec	Intensity of radiation (in ɸ/sec/ml)
0,02 M ATP	0,2	0,49±0,22 (200) $P<0,05$	—
	3,4	0,08±0,20 (200)	—
	7,0—7,5	1,26±0,19 (730) $P<0,001$	200
	9,0—9,5	7,28±0,69 (100) $P<0,001$	1100
	9,5—10,5	9,58±0,58 (60) $P<0,001$	1500
	10,5—11,0	9,43±1,48 (120) $P<0,001$	1500
	11,0—11,5	8,98±1,25 (140) $P<0,001$	1400
	11,5—12,5	7,38±0,84 (60) $P<0,001$	1100
0,02 M AMP	6,2	—0,4±0,18 (40)	—
	9,5—10,5	0,96±0,57 (80)	—
	10,5—11,5	0,45±0,87 (120)	—

Legend: number of 10-sec measurements in parentheses.

TABLE 2. Transmission of Radiation from 0.02 M ATP Solutions with Alkaline pH Values by Filters

Filter	Wave-length (in nm)	% Transmission
UFS-1	390	0
FS-6	440	25,6
SS-2	450	58,0
ZhS-16	490	40,0
OS-12	520	0

For comparison, measurements of radiation of AMP solution of the same concentration are given in Table 1; in this case no radiation was present. The data given in Table 1 reflect the mean intensity of the radiation during the first 10-15 min after preparation of the solution and the beginning of the measurements. The intensity of the radiation for ATP solutions with high pH values fell fairly sharply with time. The rate of fall of intensity, moreover, increased with an increase in pH. For instance, at pH 11.5-12.5 the intensity fell by half within the course of 30 min, at pH 9.5-10.5 it fell by 25% during the same period, but at pH 7.0-8.0 it remained virtually unchanged.

The relatively high intensity of radiation of the ATP solution at alkaline pH values enabled its approximate spectral range to be determined with the aid of filters. Filters were fixed to the movable frame instead of the quartz and glass discs. The solutions used for this purpose varied in pH from 9.5 to 11.5. Values of the radiation transmitted by the filters, in percentages of the total intensity of radiation measured without the filter, are given in Table 2.

Considering that the spectral characteristic curve of the PEM had a maximum at 340 nm, falling off sharply on the long-wave side (from 70% at 400 nm to 10% at 600 nm) it can be concluded that the region of emission of ATP is concentrated within the range 400-500 nm.

The chemiluminescence of an aqueous solution of ATP, concentrated in the visible region of the spectrum, is not the result of the spontaneous hydrolysis of the terminal phosphate group, for it is absent in an acid medium. This radiation is most probably connected with the presence of excited molecules in the solution and with intramolecular changes in their electronic structure. The proportion of these molecules in the solution, moreover, is extremely small for the quantum yield at neutral pH values is 10^{-16}-10^{-17}, but at alkaline pH values it is an order of magnitude higher. The possibility cannot be ruled out that chemiluminescence in the ATP solution may be due, for example, to the oxidation of ATP molecules by oxygen or to formation of complexes by them with metals. The possibility that such high excitation values of the ATP molecules may arise in rare cases, when they bring about the production of photic quanta, is interesting from the standpoint of an explanation of the mechanism of ATP function.

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