LIGHT AND ULTRASONIC REGULATION OF α-CHYMOTRYPSIN CATALYTIC ACTIVITY

Proflavin as a light- and sound-sensitive competitive inhibitor

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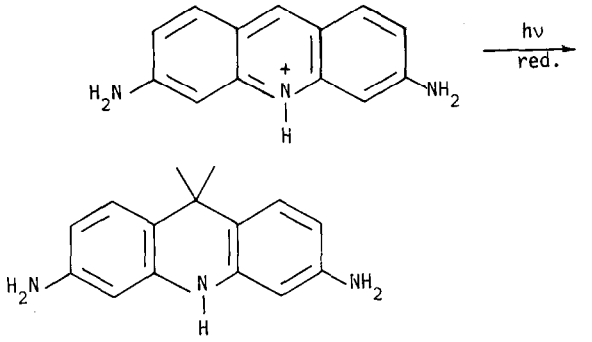
Regulation of the activity of catalysts allows chemical and biological systems to be affected in a selective manner. We are now engaged in elaborating the means of regulating the activity of biocatalysts by the action of the external field [1–3]. For instance, light initiation of the catalytic activity of the enzyme ('generation' of the active centre under the action of light) makes it possible to obtain extremely high quantum yields of the products of the conjugated catalytic reaction [3]. The present paper reports the data on the light and ultrasonic regulation of the catalytic activity of α-chymotrypsin involving a sensitive competitive inhibitor. Proflavin, an acridine dye, is one of the most effective competitive inhibitors of α-chymotrypsin [4–6]. Below it will be demonstrated how the structure of the dye can be changed, both in the photochemical and the sonochemical reaction, thereby regulating its inhibiting ability in relation to the active centre of the enzyme.

Proflavin as a light-sensitive competitive inhibitor

The thiazine and acridine dyes are photoreduced into colourless leucoforms in the presence of weak reducers [7]. We suggest that this phenomenon be used for light regulation of the inhibiting properties of proflavin ($K_i = 2 \times 10^{-5}$ M, see [4–6]). 3,6-Diaminoacridane is the product of the photochemical reduction of proflavin [8]; this substance, unlike proflavin is not flat (carbon atom C_9 becomes tetrahedrical):



We have found that the product of the photoreduction of proflavin does not possess (when the concentration of the latter is as much as 2×10^{-4} M) the properties of the competitive inhibitor of the α-chymotrypsin catalysed reactions. This means that the inhibiting ability of proflavin is at least ten times greater than that of its photoreduction product.

In fig. 1 it is shown how the catalytic activity of α-chymotrypsin changes during the photoreduction and backward oxidation of proflavin. Curve *a* shows a decrease in the concentration of proflavin, when the solution of the dye and ascorbic acid was illuminated with visible light. It can be seen that, as a result of the illumination, the concentration of proflavin decreases and achieves a stationary value (in the steady-state the rate of photoreduction is equal to that of the backward oxidation to proflavin). As the concentration of proflavin decreases, that of the free enzyme in

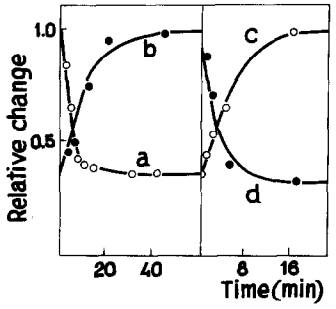


Fig. 1. Photoregulation of α-chymotrypsin activity by means of proflavin, a light-sensitive competitive inhibitor. Photoreduction of proflavin: (*a*) A decrease in absorbance of the proflavin solution, on irradiation by visible light in anaerobic conditions, and (*b*) the corresponding increase in the enzyme activity. Backward oxidation; (*c*) an increase in absorbance of the proflavin solution, on oxidation with oxygen from air, and (*d*) the corresponding decrease in the enzyme activity. Conditions: pH 4.0; 0.5 M sodium acetate; concentration of proflavin – 0.2 mM; concentration of ascorbic acid – 20 mM. Photoreduction of proflavin was carried out in the absence of the enzyme. The relative catalytic activity was measured using *p*-nitrophenyl ester of heptanoic acid as a substrate, $[S]_0$ = 0.1 mM, see [12].

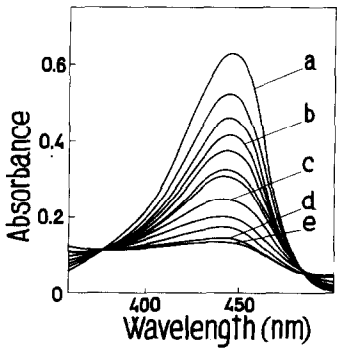


Fig. 2. The changes in the absorption spectra of the proflavin solution during sonication. Times of sonication (min): *a* = 0; *b* = 40; *c* = 80; *d* = 160; *e* = 180. Ultrasonic intensity 2 w/cm^2; frequency – 880 kHz.

the solution increases and the rate of the substrate hydrolysis goes up (fig. 1, curve *b*). The conversion of proflavin is reversible. 3,6-Diaminoacridane, formed in light, in the presence of oxygen from air backward oxidises in the dark into the proflavin. This is reflected in curve *c*, fig. 1, which shows the increase in absorbance of the photoreduced solution of proflavin as air is passed through it. The absorption spectrum of the product obtained as the result of oxidation, is similar to that of the original proflavin. As the concentration of proflavin increases, the rate of the enzyme reaction decreases (curve *d*). Complete conversion of 3,6-diaminoacridane to proflavin entails a decrease in the enzyme activity to the initial level.

Proflavin as a sound-sensitive competitive inhibitor

We found that the irreversible decolourisation of the dye takes place, when the aqueous solution of proflavin is treated ultrasonically. Fig. 2 shows how the absorption spectra of proflavin change during the ultrasonic treatment of the dye solution. The conversion of proflavin in the ultrasonic field seems to be, in some ways similar to the well-known reaction of methylene blue [9]. We have found that the products of the sonochemical reaction of proflavin do not inhibit the enzymic activity of α-chymotrypsin.

It is known that when the enzymes are treated with ultrasonics whose intensity is higher than the cavitation threshold, irreversible losses in their catalytic activity are observed [10,11]. We have chosen the experimental conditions so as to make the rate of the irreversible inactivation of the catalyst much lower than that of the sonochemical reaction of proflavin. This enabled us to observe the effect of the increasing rate of the chymotryptic hydrolysis of the substrate as a result of sonication of the enzyme-inhibitor system. Curve *a* in fig. 3 is a plot of the decreasing concentration of proflavin versus the sonication time. As the concentration of the competitive inhibitor goes down (curve *a*) the enzyme activity of the solution increases (curve *b*).

Summing up the above said, it may be concluded that with the aid of photochemical and sonochemical reactions involving the competitive inhibitors, it is possible to change the steady-state concentration of the free enzyme in the solution, thereby implementing effective regulation of the enzyme process by light and ultrasonics. It is quite possible that the methods for regulating enzyme reactions we have suggested will find application in bioengineering, particularly in connection with the use of immobilized enzymes in biochemical technology, see [13,14].

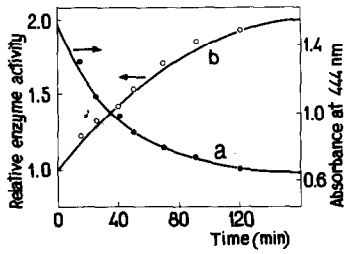


Fig. 3. Ultrasonic regulation of α-chymotrypsin activity by means of proflavin, a sound-sensitive competitive inhibitor. (*a*) A decrease in absorbance of the proflavin solution, depending on the time of ultrasonic irradiation of the dye solution in the presence of α-chymotrypsin. (*b*) The corresponding increase in the enzyme activity on ultrasonic irradiation of the α-chymotrypsin solution in the presence of proflavin. The enzyme activity was registered by a pH-stat measuring the stationary rate of hydrolysis of *N*-acetyl-L-norvaline ethyl ester, $[S]_0$ = 2.5 mM, $[E]_0$ = 3.9 M, pH 7.0, 0.058 M KCl, 15°C, see [12].

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