LIGHT-INITIATED ENZYMIC ACTIVITY CAUSED BY PHOTOSTEREOISOMERIZATION OF CIS-4-NITROCINNAMOYL-α-CHYMOTRYPSIN

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1. Introduction

According to Einstein's photochemical equivalence law restricting primary alterations caused by light in a chemical system, each absorbed light quantum can activize only one molecule of a given substance. However, the light-induced change in the system can be intensified through conjugated secondary chemical processes. Photoinitiated chain reactions may serve as examples of such reactions whose quantum yield of products is essentially more than 1. Limitation of the quantum yield value of a photoinitiated chain reaction is caused by the processes of annihilation of high-labile intermediates. This is, fortunately, not the case with light-sensitive systems in which a stable catalyst is formed under the action of light. The purpose of this communication is to discuss such a mechanism for intensifying the light effect by means of a chemical reaction, the mechanism being based on a photoinitiated catalytic process.

Systems were recently described in which an enzymic process, in itself insensitive to light, could be made subject to photoregulation [1–5]. Levels of enzyme activity could be photo-regulated by means of an 'on-or-off' assay [2]. This approach involving light-initiation of enzyme activity is of paramount importance for the intensifying light effect. We have shown [2] that the light effect on a fully inactive and relatively stable acylenzyme (*cis*-cinnamoyl-α-chymotrypsin) leads to its photostereoisomerization with subsequent formation of labile *trans*-cinnamoyl-α-chymotrypsin. The latter spontaneously deacylates to form a free enzyme. Unfortunately, this system has a substantial drawback because the protein and the chromophore *cis*-cinnamoyl group UV absorption bands considerably overlap. The sensitivity of the system in regard to the spectral composition of light can be altered by introducing a substituent into the cinnamoyl group. The present communication describes *cis*-4-nitrocinnamoyl-α-chymotrypsin (*cis*-NCCT), a light-sensitive acylenzyme which possesses suitable spectral properties for the above purpose.

2. Experimental

The materials used were described previously [3, 4]. Prior to [3] the *p*-nitrophenyl ester of *cis*-4-nitrocinnamic acid (*cis*-NPNC) was isolated from an acetonitrile solution of a *cis*- and *trans*-isomer photostationary mixture by means of thin-layer chromatography on Silufol (cyclohexane–ether = 1:5, R_f values = 0.94 and 0.82 for *trans*- and *cis*-isomers, respectively). *cis*-NCCT was obtained by reacting α-chymotrypsin with *cis*-NPNC. The experimental conditions are described in the legend to fig. 1. Acylenzyme was purified by means of gel filtration on Sephadex G-25 (pH 3.0, HCl).

Kinetic measurements were similar to those described previously [2–4]. The $k_{3(trans)}$ value, equal to k_{cat}, for nitrophenyl esters [6, 7], was determined under conditions of stationary α-chymotryptic hydrolysis of *trans*-NPNC, with $[S]_0 \gg K_{m(app)}$ (where $K_{m(app)} < 10^{-5}$ M). The rate of *p*-nitrophenolate ion liberation was followed spectrophotometrically (400 nm) at $[S]_0 = 1.7 \times 10^{-5}$ M; $[E]_0$ was varied from 7×10^{-8} M to 2×10^{-7} M.

Deacylation kinetics of *cis*-NCCT, i.e. $k_{3(cis)}$, were followed by the rate of appearance of the free enzyme: an aqueous solution of *cis*-NCCT ($\sim 10^{-5}$ M)

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was incubated in the pH-stat cuvette in the dark and samples were taken. The enzymatic activity was measured by standard procedure involving *N*-acetyl-L-tyrosine ethyl ester (using a pH-stat) [8].

A ferrioxalate actinometer was used to determine quantum yield of *cis–trans* stereoisomerization of NCCT (i.e. the quantum yield of formation of the active enzyme as a result of *cis*-NCCT irradiation was actually determined).

3. Results and discussion

It follows from fig. 1a that interaction of α-chymotrypsin and *cis*-NPNC results in enzyme inactivation. *cis*-NCCT formed as a result of the reaction, is a stable acylenzyme. For comparison, the rate constants of deacylation of both NCCT stereoisomers are given in table 1. Previously [3] we had already shown that *cis*- and *trans*-stereoisomers of NPNC considerably differ in reactivity of interaction with α-chymotrypsin. The acylation rate constants ratio has been found $(k_2/K_s)_{trans}/(k_2/K_s)_{cis} \approx 5 \times 10^3$ [3]. As seen from the table, the deacylation rate constants of the respective stereoisomers of NCCT differ to the same extent.

Table 1
The rate constants of NCCT deacylation[a].

$k_{3(cis)}$[b] (sec^{-1})	$k_{3(trans)}$[c] (sec^{-1})	$\frac{k_{3(trans)}}{k_{3(cis)}}$
$(1.7 \pm 0.5) \times 10^{-5}$	0.42 ± 0.02	2.5×10^{-4}

[a] 20 °, pH 8.0, 6.7 vol. % of dimethylsulfoxide; 0.1 M KCl.
[b] pH-stat.
[c] spectrophotometrically, 0.01 M KH_2PO_4 buffer.

Fig. 2 shows the UV absorption spectrum for *cis*-NCCT (curve a). For comparison, the same figure shows the α-chymotrypsin absorption spectrum (curve b). It is apparent that the respective absorption bands of the protein and the chromophore group are sufficiently resolved. UV irradiation (313 nm) of the *cis*-NCCT solution leads to a photochemical *cis–trans*-isomerization of the protein–chromophore compound with subsequent formation of a labile *trans*-stereoisomer, rapid deacylation of the latter results in the regeneration of free enzyme. Fig. 1b shows the increase of concentration of the free enzyme in the solution, caused by *cis*-NCCT irradiation. As is seen from fig. 1, *cis*-NCCT irradiation results in complete regeneration of the enzyme activity.

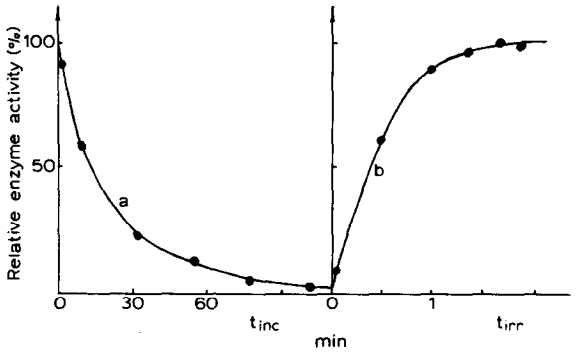

Fig. 1. Decrease in enzymic activity of α-chymotrypsin solution (5×10^{-6} M) depending on incubation time (t_{inc}) with *p*-nitrophenyl ester of *cis*-4-nitrocinnamic acid (7×10^{-5} M, curve a). The reaction was run in a pH-stat at pH 7.4, 20°, 0.33 M $Ca(NO_3)_2$ and 10 vol. % of dimethylsulfoxide. The subsequently formed *cis*-nitrocinnamoyl-α-chymotrypsin solution was irradiated with UV light at 313 nm, resulting in enzymic activity increase (curve b), the latter depending on irradiation time (t_{irrad}).

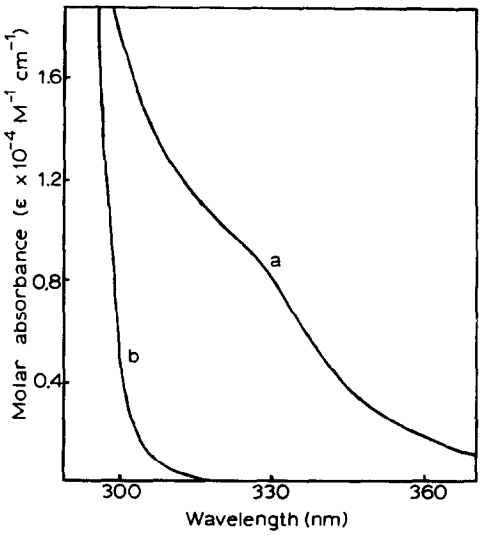

Fig. 2. UV absorption spectrum for *cis*-4-nitrocinnamoyl-α-chymotrypsin (curve a) and α-chymortypsin (curve b) at pH 7.4, 0.033 M $Ca(NO_3)_2$, 20°.

The enzyme formed by the effect of light (eq. 1a) may in the presence of a substrate bring about a substantial chemical change in the system and thereby intensify the influence of light. The quantum yield (γ) of product (P) in the 'dark' stage (1b) of the photoinitiated enzymic reaction (1a)–(1b) is, in fact, a measure of intensification of the light signal.

$$\textit{cis}\text{-NCCT} \underset{\text{UV light}}{\rightleftharpoons} \textit{trans}\text{-NCCT} \xrightarrow{k_{3(trans)}} \text{E} + \textit{trans}\text{-CA} \qquad (1a)$$

$$\text{E} + \text{S} \rightleftharpoons \text{ES} \xrightarrow{k_{cat}} \text{E} + \text{P} \qquad (1b)$$

The γ-value depends on the kinetic characteristics of the 'dark' process (1b), and may be represented (on saturating the enzyme with a substrate) as:

$$\gamma = \frac{[\text{P}]}{n} = \varphi \times k_{cat} \times t, \qquad (2)$$

where n is the number of light quanta absorbed by the system; φ is the quantum yield in the primary photochemical reaction of enzyme formation (e.g. a reaction of the 1a type); and t is the reaction time.

The k_{cat} values for the proteolytic enzyme of α-chymotrypsin reach 10^2-10^3 sec^{-1} [9]. The φ-value, measured at 313 nm (20°; pH 7.5), is normal for the *cis–trans*-isomerization process and is equal to 0.1. Thus, the quantum yield of the product of the photoinitiated enzymic reaction (1a)–(1b) over 10 min, in agreement with (2), amounts to $\gamma \sim 10^5$.

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