Biochimica et Biophysica Acta, 944 (1988) 177–184
Elsevier

BBA 74138

Photoinactivation of the thiamin transport system in *Saccharomyces cerevisiae* with azidobenzoyl derivatives of thiamin

Kenji Sempuku

Department of Biochemistry, Kyoto Prefectural University of Medicine, Kamikyoku, Kyoto (Japan)

(Received 18 January 1988)
(Revised manuscript received 6 May 1988)

Key words: Photoinactivation; Thiamin transport; Azidobenzoyl derivative; (*S. cerevisiae*)

In an attempt to obtain a potent inhibitor for thiamin transport of *Saccharomyces cerivisiae* three novel thiamin derivatives having an arylazido substituent in the thiazole moiety have been synthesized. The derivatives prepared were 4-azidobenzoylthiamin (ABT), 4-azidobenzoylthiamin disulfide (ABTD), and 4-azido-2-nitrobenzoylthiamin disulfide (ANBTD). Among the newly prepared photoreactive azidobenzoyl derivatives of thiamin, ANBTD showed the strongest competitive inhibition with an apparent K_i of 7.9 nM against thiamin uptake by *S. cerevisiae* IFO-2375. The K_i values for ABT, 4-azido-2-nitrobenzoylthiamin (ANBT), and ABTD were 187 nM, 83 nM, and 15 nM, respectively. When exposed to visible light, ANBTD inactivated in a time- and concentration-dependent manner the uptake of [^{14}C]thiamin by yeast protoplasts as well as intact cells. Remaining activities of the thiamin uptake by the intact cells were 71.9%, 27.3%, 40.1%, and 15.0% after visible light irradiation for 15 min in the presence of 1 μM ABT, ANBT, ABTD, and ANBTD, respectively. The inactivation by ANBTD (0.05 μM) was partially prevented by previous addition of an excessive amount of thiamin (5 μM). Furthermore, it was found that ANBTD (0.5 μM) irreversibly inactivated 70.6% of the thiamin-binding activity of the membrane fraction from *S. cerevisiae* IFO-2375. These results suggest that ANBTD can inhibit yeast thiamin transport by photoinactivation of membrane-bound thiamin-binding protein in the plasma membrane which may be a functional component involved in the thiamin transport system of *S. cerevisiae*.

Introduction

Previously it has been demonstrated that thiamin is accumulated in large amounts in *Saccharomyces cerevisiae* cells by a carrier-mediated active transport process [1,2]. The protein components playing the uptake role in the cell membrane have, however, remained unclear until the present time. Recently, the thiamin-binding activity has been found in both the soluble and membrane portions of yeast [3] and a soluble thiamin-binding protein has been isolated from osmotically shocked fluids of yeast cells [4]. A more recent study has shown that *S. cerevisiae* protoplasts accumulate thiamin in the same manner as the intact cells [5], suggesting that the soluble thiamin-binding protein in the yeast periplasm may not be directly involved in the thiamin transport system. Furthermore, it has been disclosed that the thiamin-binding protein located in the plasma membrane of *S. cerevisiae* plays an important role in thiamin uptake [6].

Meanwhile the thiamin transport system [7,8] has been studied by the use of chemical compounds which interact with the thiamin transport

---

Correspondence: K. Sempuku, Department of Biochemistry, Kyoto Prefectural University of Medicine, Kamikyoku, Kyoto 602, Japan.

system in *S. cerevisiae*. Among them, 4-azido-2-nitrobenzoylthiamin (ANBT), a water-soluble azidobenzoyl derivative of thiamin, has proven to be a specific irreversible photoreactive inhibitor for the thiamin transport system in yeast cells [8].

The specific irreversible binding of chemical compounds to functional proteins has generally been one of the useful means of detecting transport carrier components in biomembranes [9].

The present paper describes the synthesis of novel azidobenzoyl derivatives of thiamin, including 4-azido-2-nitrobenzoylthiamin disulfide (ANBTD), an exceedingly potent inhibitor with lipophilic character, their inhibition of thiamin uptake by yeast cells, and the photoinactivation of the thiamin transport system in the intact cells and protoplasts and of thiamin-binding activity of the yeast membrane fraction using these derivatives.

Materials

Chemicals. [*thiazole*-2-^{14}C]Thiamin hydrochloride (24.3 Ci/mol) was obtained from the Radiochemical Centre, Amersham, U.K. Thiamin hydrochloride was a commercial chemical of reagent grade. 4-Azidobenzoic acid and 4-azido-2-nitrobenzoic acid were synthesized according to the methods of Galardy et al. [10] and Lewis et al. [11], respectively. Thiamin disulfide was prepared by the method of Kawasaki et al. [12]. ANBT was prepared as previously described [8]. All other chemicals were purchased from commercial suppliers.

Organism. The strain *S. cerevisiae* IFO-2375 was kindly donated by Professor Akira Kimura, Research Institute for Food Science, Kyoto University Uji, Japan.

Methods

The synthetic and biochemical experiments were done in the dark or under subdued light when the azido compounds were being treated.

Growth of yeast cells. S. cerevisiae IFO-2375 was grown in Wickerham synthetic medium with thiamin omitted as described in a previous paper [3].

Transport assays. Transport assays of [^{14}C] thiamin were carried out according to the method of Iwashima et al. [2].

Irradiation procedure. Photoinactivation was performed by the same procedure as previously reported [8].

Photoinactivation and transport assay with yeast protoplasts. The protoplasts prepared by the procedure of Theuvenet and Bindels [13] were suspended at the concentration of 0.37 mg dry weight/ml in 2 ml of 0.05 M potasium phosphate buffer (pH 5.0) containing 3% (w/v) glucose, 0.82 M sorbitol, and 1 mM $CaCl_2$ and subjected to the above-mentioned photoinactivation procedure. Then, the suspensions were centrifuged for 5 min at 2000 × *g* and washed twice with the same buffer. The protoplast pellets were resuspended at a concentration of 0.037 mg dry weight/ml and aliquots of 0.5 ml were used for the transport assays as previously reported [5].

Assay for thiamin-binding activity of membrane fraction from photoinactivated yeast cells. Yeast cells (10 liter culture batch) were harvested, washed, and suspended in 0.05 M potassium phosphate buffer (pH 5.0) at a concentration of 1.2 mg dry weight/ml. An aliquot of 750 ml of the suspension was placed in a shallow glass dish (18 cm in diameter), ice-cooled, stirred, and irradiated for 15 min with a Toshiba black light lamp (40 watts) from a distance of 25 cm after addition of $0.5 \cdot 10^{-6}$ M of ANBTD. The preparation of membrane fractions of *S. cerevisiae* was carried out by sonication followed by differential centrifugation according to the procedure previously reported [3]. The membrane fraction was suspended in 0.05 M potassium phosphate buffer (pH 5.0) and subjected to equilibrium dialysis against 10^{-7} M [^{14}C]thiamin overnight in the cold for the assay of thiamin-binding activity.

Synthesis of 4-azidobenzoylthiamin (ABT). 4-Azidobenzoic acid (4.2 g, 25.7 mmol) was heated with thionyl chloride (10 ml) for 75 min under reflux, concentrated under vacuum at 50 °C, and traces of excess thionyl chloride were removed by addition of benzene and repeated evaporation to leave crystalline 4-azidobenzoyl chloride, which was then used without further purification. To the crystalline residue were added dried thiamin hydrochloride (2.9 g, 8.6 mmol) and dry pyridine (10 ml) and the resulting slurry was heated for 60 min at 90 °C. The reaction mixture was concentrated under vacuum at 90 °C, the residue was triturated with acetone, filtered, and washed well with acetone. Recrystallization of the powdery product from methanol containing 5% water gave colorless needles of 4-azidobenzoylthiamin hydrochloride monohydrate (1.9 g), m.p. 189 °C (decomposed), soluble in water.

$C_{19}H_{20}ClN_7O_2S \cdot HCl \cdot H_2O$ (M_r 500.40)
Calcd.: C, 45.61; H, 4.63; N, 19.59
Found: C, 45.24; H, 4.60; N, 19.62

Infrared (KBr) 2120 cm^{-1} (azido), 1715 cm^{-1} (carbonyl). Ultraviolet (in pH 2 aqueous HCl) λ_{max} 270 nm ($\epsilon = 22100$).

Synthesis of 4-azidobenzoylthiamin disulfide (ABTD). 4-Azidobenzoic acid (522 mg, 3.2 mmol) was converted into the chloride form in the same manner as in the synthesis of ABT. To the chloride were added thiamin disulfide (450 mg, 0.8 mmol) and dry pyridine (3 ml) and the resulting solution was allowed to stand for 3 days at room temperature, concentrated under vacuum at 40 °C, the residue was washed with 10% aqueous Na_2CO_3 in chloroform, and the chloroform layer was dried over anhydrous Na_2SO_4 and evaporated. The gummy residue was triturated and crystallized with diethyl ether to give a crude product (480 mg) containing some unreacted 4-azidobenzoic acid. Part of this was dissolved in a small amount of tetrahydrofuran, streaked onto preparative thin layer plates (20 × 20 cm, 5 mm in thickness) of silica gel, and developed with 20 : 1 (v/v) chloroform/methanol. The lowest band sensitive to ultraviolet rays (2536 Å) was scraped off and extracted with methanol, the extract was filtered, the filtrate was concentrated, and the crystalline residue was recrystallized from ethanol to give colorless needles, m.p. 162 °C.

$C_{38}H_{40}N_{14}O_6S_2$ (M_r 852.96)
Calcd.: C, 53.51; H, 4.73; N, 22.99
Found: C, 53.76; H, 4.75; N, 22.98

Infrared (KBr) 2125 cm^{-1} (azido); 1710 cm^{-1} (carbonyl). Ultraviolet (in ethanol) λ_{max} 274 nm ($\epsilon = 55000$).

Synthesis of 4-azido-2-nitrobenzoylthiamin disulfide (ANBTD). 4-Azido-2-nitrobenzoic acid (333 mg, 1.6 mmol) was converted to the chloride [8]. To this were added thiamin disulfide (225 mg, 0.4 mmol) and dry pyridine (1.5 ml) and the resulting solution was allowed to stand overnight at room temperature and concentrated under vacuum at 40 °C. The dark-colored residue was subjected to column chromatograph (4 × 6 cm) of silica gel in 5 : 1 (v/v) chloroform/methanol to give, after evaporation of the first 200-ml run, a pale yellow powdery crude ANBTD weighing 340 mg. Part of this was dissolved in a small amount of tetrahydrofuran, applied to preparative thin layer plates (20 × 20 cm, 5 mm in thickness) of silica gel, and developed with 5 : 1 (v/v) chloroform/methanol. A band ranging from R_F 0.5 to R_F 0.7 sensitive to ultraviolet rays of 2536 Å was scraped off and extracted with methanol. The extract was filtered, the filtrate was concentrated, and the crystalline residue was recrystallized from ethanol/tetrahydrofuran to give colorless needles, m.p. 180 °C.

$C_{38}H_{38}N_{16}O_{10}S_2$ (M_r 942.98)
Calcd.: C, 48.40; H, 4.06; N, 23.77
Found: C, 48.37; H, 4.16; N, 23.98

Infrared (KBr) 2120 cm^{-1} (azido); 1725 cm^{-1} (carbonyl); 1540 cm^{-1} (nitro). Ultraviolet (in ethanol) λ_{max} 244 nm ($\epsilon = 49500$); λ_{max} 272 nm ($\epsilon = 42000$).

Reduction of ABTD [12]. A mixture of ABTD (2 mg) and cysteine hydrochloride (2 mg) in 50% aqueous ethanol was heated at 50 °C. In the course of 1 h the suspension turned to a solution, which soon gave a crystalline precipitate. After addition of a small amount of water, an aliquot of the resulting solution was subjected to thin layer chromatography on silica gel in *n*-propanol/sodium acetate buffer (1 M, pH 5.0)/water (65 : 15 : 20, v/v) [14] together with ABT as reference. Under iodine vapor the R_F was shown to be 0.26, which was identical to that of the reference sample of ABT, whereas the R_F of starting disulfide (ABTD) was 0.58.

Reduction of ANBTD [12]. A treatment similar to the above with ANBTD as starting material gave a reaction product which showed an R_F of

0.22 corresponding to that of ANBT. The R_F of starting disulfide (ANBTD) was 0.54.

Results

Synthesis of photoreactive azidobenzoyl derivatives of thiamin

In addition to ANBT which was prepared in the earlier study [8], three novel thiamin derivatives having an arylazido substituent in the thiazole moiety were synthesized as inhibitors for the thiamin transport system in yeast. Chemical structures of the compounds synthesized so far are illustrated in Fig. 1. The synthesis was carried out by esterification of thiamin itself or the dimer, thiamin disulfide, with substituted benzoyl chlorides under basic conditions. Lipophilic dimeric ABTD and ANBTD were separated and purified from the reaction mixtures by means of preparative thin layer chromatography in organic solvents. Reduction of ABTD and ANBTD with cysteine gave ABT and ANBT, respectively, thus confirming the structural relationship between the dimers and monomers within the four thiamin derivatives. Infrared measurement showed a sharp absorption band by the azido group in all the compounds at 2120–2125 cm^{-1}. A shift in the ultraviolet absorption maximum toward shorter wavelength by approx. 20–30 nm was observed in ANBT and ANBTD when a nitro group was introduced into the *ortho* position of the benzene ring of ABT or ABTD [8]. This hypsochromic effect was probably due to a deviation from coplanarity of the benzene ring with the carbonyl group resulting from the occupation of the planarly outstretched *o*-nitro group.

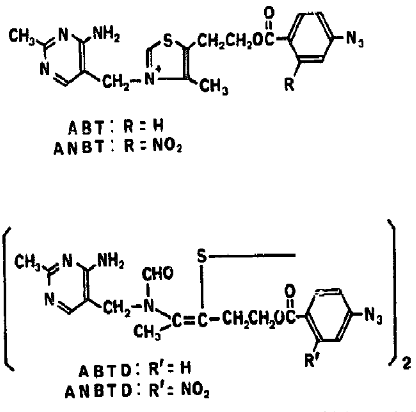

Fig. 1. Chemical structures of photoreactive azidobenzoyl derivatives of thiamin.

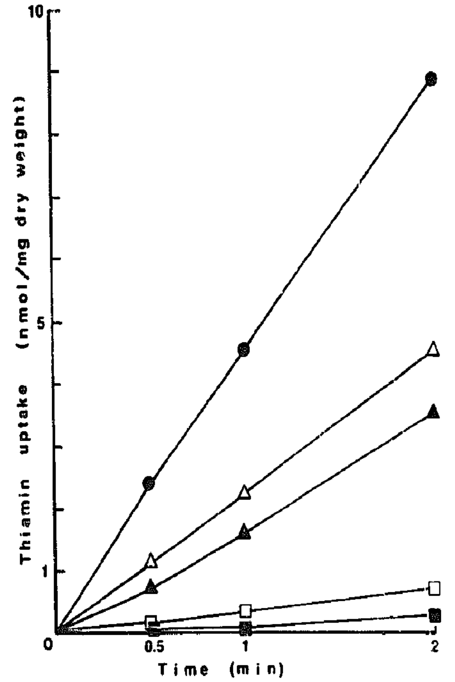

Fig. 2. Time course of $[^{14}C]$thiamin uptake in the presence of azidobenzoyl derivatives of thiamin. After preincubation for 15 min at 37° C, yeast cell suspensions (37 μg dry weight/ml) were exposed simultaneously to 1 μM $[^{14}C]$thiamin and 1 μM ABT, ANBT, ABTD, or ANBTD for 2 min at 37° C. Each point represents the mean of three experiments. Control (●), ABT (△), ANBT (▲), ABTD (□), ANBTD (■).

Competitive inhibition of yeast thiamin transport by azidobenzoyl derivatives of thiamin

Fig. 2 shows the time course of $[^{14}C]$thiamin uptake inhibited by ABT, ANBT, ABTD, or ANBTD upon incubation for 2 min at 37° C. The transport of thiamin in the presence and absence of the inhibitors proceeded linearly within the employed range of time. Moderate inhibitions were observed with ABT and ANBT, whereas considerably strong inhibitions were seen with the lipophilic dimeric thiamin derivatives ABTD and ANBTD. The K_i values for the four azidobenzoyl derivatives of thiamin were given by the kinetics of thiamin transport for 1 min at 37°C after preincubation of the cell suspensions for 15 min. The pattern obtained in double-reciprocal plots for ANBTD is shown in Fig. 3. ANBTD exerted competitive inhibition with [^{14}C]thiamin and the K_i value of 7.9 nM was obtained from linear plots of slope versus [^{14}C]thiamin at various concentrations of ANBTD. For ABT, ANBT, and ABTD apparent K_i values obtained in the same manner as above were 187 nM, 83 nM, and 15 nM, respectively. An apparent K_t of thiamin for *S. cerevisiae* IFO-2375 calculated from a Lineweaver-Burk plot was found to be 0.67 μM. Since the K_i value for *O*-benzoylthiamin disulfide, the most potent competitor against thiamin so far discovered, is 1.8 nM in yeast cells [15], it appears that ANBTD in this series is another remarkably potent competitive inhibitor and the potency is approx. 10-fold higher than that of the corresponding monomer, ANBT.

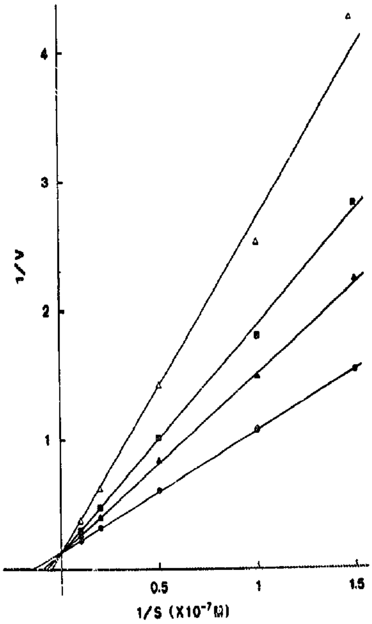

Fig. 3. Competitive inhibition of thiamin uptake by ANBTD. After preincubation for 15 min at 37°C, yeast cell suspensions (37 μg dry weight/ml) were incubated for 1 min with various concentrations of [^{14}C]thiamin in the presence of none (●), 5 nM (▲), 10 nM (■), and 15 nM (△) ANBTD. The reciprocal velocities were plotted against the reciprocal [^{14}C]thiamin concentrations. Each point represents the mean of three experiments.

Photoinactivation of thiamin transport in intact cells and protoplasts of yeast by azidobenzoyl derivatives of thiamin

Before the photoinactivation study was carried out, the optimal time span for visible light irradiation was examined. It was found that 15 min was satisfactory for this purpose (Fig. 4) when the yeast cells were treated with 10^{-6} M ANBTD followed by uptake assay with 10^{-6} M [^{14}C]thiamin. Remaining activities were 46%, 29%, and 24% after 2 min, 5 min, and 10 min, respectively, in the course of irradiation, reaching a plateau of 17–18% after 15 min as shown in Fig. 4.

Fig. 5 shows the decrease of [^{14}C]thiamin uptake by yeast cells photochemically treated for 15 min with ABT, ANBT, ABTD, or ANBTD at various concentrations as compared to that of the cells without irradiation. Photoinactivation occurred, as expected, moderately with ABT and most strongly with ANBTD; ANBT and ABTD showed inhibitions between those of these two inhibitors. Remaining activities of the thiamin uptake by the intact yeast cells were 71.9%, 27.3%, 40.1%, and 15.0% after 15 min visible light irradiation in the presence of 10^{-6} M ABT, ANBT, ABTD, and ANBTD, respectively. The values were 89.7%, 39.7%, 50.0%, and 23.7% after treatment with $0.5 \cdot 10^{-6}$ M ABT, ANBT, ABTD, and ANBTD, respectively. At concentrations as low as 10^{-7} M, ANBTD still eliminated 66.4% of the transport activity from yeast cells after thorough washing of the irradiated cell suspension with 0.05 M potassium phosphate buffer (pH 5.0) (Table I). The degree of inactivation was dependent on the concentrations of the inhibitors (Fig. 5). On the other hand, treatment of the cell suspensions with 10^{-4} M each of the azido derivatives for 15 min in the dark and subsequent washing with 0.05 M potassium phosphate buffer (pH 5.0) retained the original full thiamin uptake activity in all cases, and simple irradiation in the absence of the inhibitors did not influence the transport activity to any degree (data not shown). These results indicated that visible light irradiation in the presence of the azido compounds would essentially give rise to

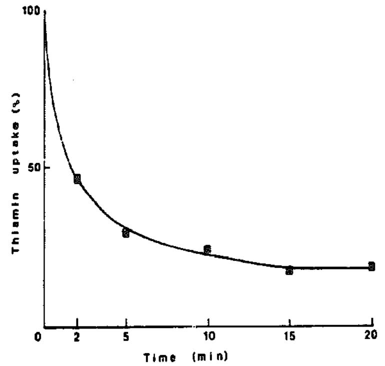

Fig. 4. Time course of photoinactivation of [^{14}C]thiamin uptake by ANBTD. Yeast cell suspensions (0.37 mg dry weight/ml) were irradiated as described in Methods, and then subjected to transport assay after the washing of cells with buffer. Remaining activity of [^{14}C]thiamin uptake is expressed as a percentage of original full activity. Each point represents the mean of two experiments.

TABLE I

ANBTD-DEPENDENT PHOTOINACTIVATION OF THIAMIN TRANSPORT IN INTACT YEAST CELLS AND PROTOPLASTS

Intact yeast cells and protoplast preparation, photoinactivation, and thiamin transport assay were carried out as described in Methods. Values are averages of three independent experiments.

Concn. of ANBTD (μM)	Thiamin uptake (%)	
	intact cells	protoplasts
0	100.0	100.0
0.05	38.0	40.4
0.1	33.6	35.8
0.5	23.7	20.0
1	15.0	11.3

probable chemical covalent bonding between the azido group and some protein components involved in the thiamin transport in yeast through photogenerated arylnitrene intermediates. A reference assay was exercised in the presence of a relatively high concentration (5 μM) of thiamin to determine whether thiamin might protect the yeast thiamin transport system from inactivation by ANBTD (0.05 μM). The result showed that thiamin, added simultaneously at a concentration 100-fold that of ANBTD, prevented the inactivation by 67.9% for the intact cells of yeast (Table II).

An earlier study in this laboratory demonstrated that thiamin is accumulated in protoplasts of *S. cerevisiae* in the same manner as in the intact cells [5]. In this connection, *S. cerevisiae* protoplasts were subjected to the same photolysis in the presence of 10^{-6} M ANBTD followed by [^{14}C]thiamin uptake assay. The activity was decreased as the concentration of ANBTD increased

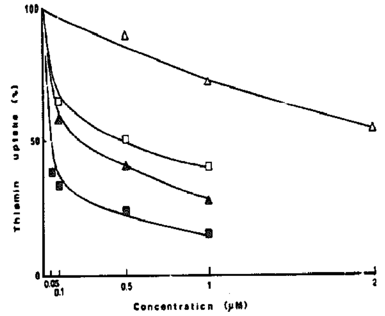

Fig. 5. Photoinactivation of [^{14}C]thiamin uptake by various concentrations of azidobenzoyl derivatives of thiamin. Photoinactivation studies were carried out as described in Methods with or without thiamin derivatives at indicated concentrations under visible light irradiation. Remaining activity of [^{14}C]thiamin uptake is expressed as a percentage of original full activity. Each point represents the mean of three experiments. ABT (△), ANBT (▲), ABTD (□), ANBTD (■).

TABLE II

SPECIFIC PREVENTION OF ANBTD-DEPENDENT PHOTOINACTIVATION BY THIAMIN IN YEAST THIAMIN TRANSPORT

Intact yeast cells and protoplast preparation, photoinactivation, and thiamin transport assay were carrier out as described in Methods. The photoinactivation was performed in the presence and absence of thiamin. Values are mean ± S.E. from five independent experiments. Significant difference from no addition of thiamin, $P < 0.01$.

Concn. of ANBTD (μM)	Thiamin (5 μM)	Thiamin uptake (%)	
		intact cells	protoplasts
0	-	100.0	100.0
0.05	-	36.9±6.3	39.4±4.4
0.05	+	67.9±3.5	67.4±8.3

TABLE III

IRREVERSIBLE PHOTOINACTIVATION WITH ANBTD OF THIAMIN-BINDING ACTIVITY IN YEAST MEMBRANE FRACTION

Preparation of the yeast membrane fraction after photoinactivation were carried out as described in Methods. Reference uptake assays for whole cells was performed in parallel by sampling portions of yeast suspensions prior to sonication. Average values of two independent experiments are given.

Addition (μM)	Irradiation	Thiamin binding (%)	Thiamin uptake by whole cells (%)
No addition	+	100.0	100.0
ANBTD, 0.5	−	101.6	103.6
ANBTD, 0.5	+	29.4	27.4

from $5 \cdot 10^{-8}$ M to 10^{-6} M and the tendency was quite similar to that for intact cells (Table I). Partial prevention of the inactivation was observed also with the protoplasts; uptake was measured after irradiation in the presence of ANBTD (0.05 μM) and excess thiamin (5 μM) as in the case of the intact cells (Table II).

Photoinactivation of yeast membrane fraction by azidobenzoyl derivative of thiamin (ANBTD)

Since the results obtained with the protoplasts indicated that the probable target of ANBTD was the yeast plasma membrane, the interaction of ANBTD with membrane-bound thiamin-binding protein in the yeast membrane fraction was examined in order to obtain an insight into the details on the thiamin transport mechanism. As shown in Table III, a decrease in binding with [^{14}C]thiamin was obviously noted at the membrane level with $0.5 \cdot 10^{-6}$ M ANBTD, and the remaining thiamin-binding potency was 29.4% after irreversible photoinactivation. In an experiment performed in parallel to this, the [^{14}C]thiamin transport activity in the intact cells treated with ANBTD agreed fairly well with the thiamin-binding activity of the yeast membrane fraction, suggesting that the inactivation of membrane-bound thiamin-binding protein would result in decreased thiamin transport activity in *S. cerevisiae*. The binding activity of the membrane fraction with [^{14}C]thiamin remained unaltered after treating with $0.5 \cdot 10^{-6}$ M ANBTD when visible light irradiation was not given (Table III).

Discussion

Photosensitive arylazide compounds capable of forming covalent bonds with physiologically important components in biomembranes are valuable in studies on the functional binding sites of enzymatic proteins and polypeptides [16–18]. In order to elucidate the components of the thiamin transport system in yeast, the synthesis of ABT, ABTD, and ANBTD and their photoinactivation studies (together with previously prepared ANBT) have been carried out, investigating [^{14}C]thiamin uptake by yeast cells and protoplasts and [^{14}C]thiamin binding to the membrane fraction of yeast.

Synthesized ABT is hydrophilic and contains one molecule of hydrate water. In the series of *O*-benzoylthiamin derivatives, however, the introduction of a nitro group onto the benzene ring excludes hydrate water [8,19]. The nitro group probably diminishes hydrophilicity and enhances crystallizability for the thiamin derivative molecules. As clearly seen from the R_F values of the four azidobenzoyl derivatives, ABTD and ANBTD have exceedingly high lipophilicity and have in fact been separated and purified by means of silica gel chromatography in chloroform/methanol.

From the results of the uptake assay for thiamin, the four azidobenzoyl derivatives of thiamin can be classified in two categories in terms of inhibition. The water-soluble monomeric derivatives ABT and ANBT belong to one class with moderate inhibition, whereas the disulfide derivatives ABTD and ANBTD are classified as potent inhibitors. Furthermore, as seen from the K_i values for these derivatives, the difference in inhibitory effects between the monomers and the dimeric disulfides is dramatic. Dimeric *O*-benzoylthiamin disulfude, having no substituent on the benzene ring, also shows an extraordinarily high inhibitory potency [15]. The reason for this tendency remains uncertain, but it would seem likely that the strong compatibility of lipophilic thiamin disulfides with the lipid components in the yeast plasma membrane is responsible for these findings.

The order of inhibitory effect of thiamin derivatives, ABT, ANBT, ABTD, and ANBTD, on the thiamin uptake by photochemically treated yeast cells is interesting compared with their chemical structures. The inhibition has been potentiated by the coexistence of a nitro group and an azido group on the benzene ring. Thus, the inhibition caused by ANBT was found to be comparable to that by ABTD as shown in Fig. 5. This result seems notable because the nitro group in ANBT has compensated, in photoinactivation potency, for the lipophilicity of ABTD which is devoid of a nitro group. Observations in the present study agree with the remarks that nitroarylazido compounds are generally more easily photoactivated by visible light than arylazido compounds lacking in the nitro group [20] and that the nitroarylnitrene intermediates which are formed during photoirradiation acquire more enhanced reactivity than the corresponding nitro group-free arylnitrene compounds [21].

As shown in Table I, ANBTD also inactivated the thiamin transport system in enzymatically isolated protoplasts, i.e., the enzymatic removal of the cell wall influenced neither the uptake ability nor the inhibitory effect of ANBTD on yeast cells.

In addition, partial protection of the thiamin uptake activity both in the intact yeast cells and protoplasts by addition of excess thiamin to ANBTD (Table II) suggests that binding to the thiamin-specific component in the plasma membrane (probably thiamin carrier protein) by the arylnitrene formed from ANBTD was hindered in the presence of the thiamin.

The inhibition of [^{14}C]thiamin binding to the yeast membrane fraction by $0.5 \cdot 10^{-6}$ M ANBTD to the extent of 70.6% (Table III) indicates that the membrane-bound thiamin-binding protein described in a previous paper [5] could be directly related to the thiamin transport system in *S. cerevisiae*.

Thus, it seems probable that the thiamin binding site in the active transport system for thiamin in yeast cells is closely related to the membrane-bound thiamin-binding protein, although possible identity of the membrane-bound thiamin-binding protein with thiamin carrier protein remains to be clarified. In this context, the light-sensitive azidobenzoyl derivatives of thiamin synthesized in this study appear to be of use as photolabels for the transport site in *S. cerevisiae*.

Acknowledgements

The author wishes to acknowledge valuable advice, encouragement and critical reading of the manuscript by Professor A. Iwashima. Thanks are also due to Dr. H. Nishino and Dr. H. Nishimura in the same laboratory for their useful discussions.

References

1 Suzuoki, Z. (1955) J. Biochem. 42, 27–39.
2 Iwashima, A., Nishino, H. and Nose, Y. (1973) Biochim. Biophys. Acta 330, 222–234.
3 Iwashima, A., Nishimura, H. and Nose, Y. (1979) Biochim. Biophys. Acta 557, 460–468.
4 Iwashima, A. and Nishimura, H. (1979) Biochim. Biophys. Acta 577, 217–220.
5 Nishimura, H., Sempuku, K. and Iwashima, A. (1982) J. Bacteriol. 150, 960–962.
6 Nishimura, H., Nosaka, K., Sempuku, K. and Iwashima, A. (1986) Experientia 42, 607–608.
7 Iwashima, A., Nishino, H., Sempuku, K. and Nishimura, H. (1981) Experientia 37, 473–474.
8 Sempuku, K., Nishimura, H. and Iwashima, A. (1981) Biochim. Biophys. Acta 645, 226–228.
9 Becker, A.J., Wilchek, M. and Katchalski, E. (1971) Proc. Natl. Acad. Sci. USA 68, 2604–2607.
10 Galardy, R.E., Craig, L.C., Jamieson, J.D. and Printz, M.P. (1974) J. Biol. Chem. 249, 3510–3518.
11 Lewis, R.V., Roberts, M.F., Dennis, E.A. and Allison, W.S. (1977) Biochemistry 16, 5650–5654.
12 Kawasaki, C., Tomita, I. and Daira, I. (1962) Bitamin 25, 61–63.
13 Theuvenet, A.P.R. and Bindels, R.J.M. (1980) Biochim. Biophys. Acta 599, 587–595.
14 Neal, R.A. (1969) J. Biol. Chem. 244, 5201–5205.
15 Iwashima, A., Wakabayashi, Y. and Nose, Y. (1977) J. Bacteriol. 131, 1013–1015.
16 Schaefer, H.J., Rathgeber, G., Dose, K., Masafumi, Y. and Kagawa, Y. (1985) FEBS Lett. 186, 275–280.
17 Emerit, M.B., Gozlan, H., Marquet, A. and Hamon, M. (1986) Eur. J. Pharmacol. 127, 67–81.
18 Safa, A.R. and Felsted, R.L. (1987) J. Biol. Chem. 262, 1261–1267.
19 Unoki, M. and Mitsugi, T. (1964) Yakugaku Zasshi 84, 780–783.
20 Chowdhry, V. and Westheimer, F.H. (1979) Annu. Rev. Biochem. 48, 293–325.
21 Knowles, J.R. (1972) Acc. Chem. Res. 5, 155–160.