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Thionicotinamide-NADP, a Nucleotide Analog Interfering with Ferredoxin-NADP Reductase/Ferredoxin Interaction

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Thionicotinamide-NADP, Ferredoxin, Ferredoxin-NADP Reductase, Protein/Protein Interaction

Diaphorase and transhydrogenase activities (system NADPH; dichlorophenolindophenol and NADPH;NAD, respectively) of ferredoxin-NADP reductase are increased by ferredoxin. Reduced thionicotinamide-NADP (TN-NADPH) only slightly inhibits these activities with no ferredoxin present in the assay, but the activity increments in the presence of ferredoxin are strongly decreased. Photosynthetic pyridine nucleotide reduction is also inhibited by the reduced analog, the extent of inhibition being approx. in the same order with all three activities. The ferredoxin stimulated activities therefore appear to be due to the same interaction between reductase and ferredoxin in all three cases.

The inhibition by TN-NADPH resembles that with Na-pyrophosphate (or NaCl), although in contrast to salt inhibition it is **a.**) not alleviated by higher ferredoxin concentrations, and **b.**) it still allows reductase/ferredoxin binding.

The binding of the reduced pyridine nucleotide analog to the reductase seems to interfere rather specifically with the stimulation which ferredoxin can exert on the enzymic activities of the reductase.

In a previous paper [1] data were presented about the influence of ferredoxin on enzymatic activities of ferredoxin-NADP reductase. Diaphorase and transhydrogenase activities of this enzyme were increased severalfold by the non-heme iron protein. This stimulation of activity is due to complex formation between the two proteins. In photosynthetic NADP reduction this complex formation is also very probable. By kinetics and inhibitor studies two binding sites (synonymous with reaction sites) on

The following, non-standardized, abbreviations are used: AMP = adenosine monophosphate (adenylate), Chl = chlorophyll (a), DCIP = dichlorophenolindophenol, E = optical density (extinction), ΔE = change of optical density (per time), Fd = ferredoxin from *Bumilleriopsis filiformis* VISCHER, TN-NADP = thionicotinamide-NADP (oxygen in the carbamyl group is replaced by sulfur), oxidized form; TN-NADPH = reduced form, TRIS-HCl = tris-(hydroxymethyl)-amino methane, buffer, adjusted with HCl.

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the reductase were concluded, one for ferredoxin and (certain) diaphorase substrates, and one for pyridine nucleotides).

From these findings it was assumed that diaphorase and transhydrogenase activities of the isolated enzyme are closely related to the (physiological) photosynthetic NADP reduction or to a part of it [2, 3].

The reductase/ferredoxin complex is destroyed by salts, e. g. NaCl, $MgCl_2$ [4, 1] or sodium pyrophosphate [5, 6] and methylene diphosphate [7]. Correspondingly these salts inhibit the stimulation of diaphorase and transhydrogenase activities [1, 3]. Since the effective concentrations are rather high (more than 10 mM) these inhibitors seem to act unspecifically by their ionic strength (see Table 3 in l. c. [3]).

This paper presents experimental data showing that reduced thionicotinamide-NADP is quite a specific inhibitor of certain reductase/ferredoxin interactions.

Materials and Methods

Purification and characterization of ferredoxin-NADP reductase (EC 1.6.99.4/1.6.1.1) and ferredoxin (both from the heterokont alga *Bumilleriopsis filiformis* VISCHER) were already published in detail [1, 8, 9]. The millimolar extinction coefficients are 10.7 $mM^{-1} \times cm^{-1}$ at 458 nm for the reductase and 9.2 $mM^{-1} \times cm^{-1}$ for ferredoxin at 420 nm.

Enzyme preparations are stored at −25 °C and generally some loss of activity has occurred before use (for specific activities of freshly prepared reductase see Table, p. 323 in l. c. [1]). This does not affect, however, the increase of the enzymic activities by added ferredoxin. The diaphorase and transhydrogenase assays are performed as described [1], alterations are noted in the legends. Generally enzyme activity is expressed by the rate: change of optical density ΔE per 10 sec since the readings are taken at this time interval. Often the rates are based on an enzyme concentration equivalent to an optical density of 1 at 458 nm (i. e. the flavin maximum of the reductase). Oxygen is measured with the Au/Ag-CLARK electrode from Beckman Instruments. The teflon membrane is changed every two days. Generally NADPH is the H-donor in 0.1 mM concentration. Stimulation of activity by ferredoxin is very good at pH 7 in TRIS-HCl buffer, which is therefore used throughout although its pK is unfavourable.

When the oxidized form of TN-NADP is present in the assay it is reduced within seconds by transhydrogenase in the test (compare Fig. 1 in l. c. [2]). Generally TN-NADPH is generated in the very same assay reaction mixture before the inhibition of the particular reaction under investigation is started. In some cases TN-NADPH is reduced beforehand and separately by isocitrate, isocitrate dehydrogenase and transhydrogenase. For this purpose the nucleotide analog (2.5 mM) is included in the transhydrogenase test (see legend of Table 1) with 0.1 μM transhydrogenase (= Fd-NADP reductase) and NAD omitted. After 4 hrs. of reaction time the mixture is heated at 100 °C for 4 min. The yield of the reduced analog is between 25 and 35%.

Additions		Rates in $\Delta E_{340} \times 10^{-3}$/10 sec (in A) Rates in $\Delta E_{400} \times 10^{-3}$/10 sec (in B)		Rate (+) Fd / Rate (−) Fd
	[mM]	(−)Fd	(+) Fd	
A. with NAD as hydrogen acceptor:				
(−) inhibitor	—	9.5	28.0	3.0
2′-AMP	1.0	3.5	10.0	2.9
	2.0	2.0	5.2	2.6
3′-AMP	1.0	7.0	19.5	2.8
5′-AMP	1.0	7.8	—	—
NAD	6.0	4.8	13.0	3.1
NADPH	1.5	5.0	15.0	3.0
	5.0	0.7	2.4	3.4
sodium dodecylsulfate	0,40	5.0	14.5	2.9
digitonin	0.08	5.3	17.0	3.2
p-chloro-mercuribenzoate	0.1	3.6	5.0	1.4
	0.2	3.0	4.0	1.3
B. with TN-NAD as hydrogen acceptor:				
NADPH	0.02	15.9	37.0	2.3
	0.13	6.4	15.2	2.4
	0.50	3.9	9.0	2.3

Table 1. Influence of pyridine nucleotides and AMP on transhydrogenase activity and its increase by ferredoxin. The reaction mixture contains in μmoles in 1 ml: TRIS-HCl, pH 7.0, 80; $MgCl_2$ 2; NADP 0.01; Na-isocitrate 3; isocitrate dehydrogenase 0.19 mg/ml; NAD 1 (in A) and TN-NAD 1 (in B); Fd-NADP reductase 1.7×10^{-3} (in A) and 1.7×10^{-4} (in B); ferredoxin (= Fd), if added, 7.4×10^{-3}.

For cultivation of the alga, isolation of its chloroplasts and the assay of photosynthetic NADP reduction see l. c. [10, 11].

Results

In Table 1 some inhibitors are listed with regard to their action on transhydrogenase activity without and with ferredoxin present in the assay.

2′-AMP, but not the 3′- and 5′-isomers, and all pyridine nucleotides, whether they are reduced or oxidized, inhibit the basal rate (i. e. the rate with no ferredoxin present in the assay). – Data for NADP and NADH are not listed here, since they are noted in Figs. 3 and 4 of l. c. [2]). The stimulation factor (obtained by dividing the rate with Fd in the assay by the basal rate) however, is hardly affected. In l. c. [2] it was demonstrated that the nucleotides bind primarily at a separate enzyme site, different from the ferredoxin binding site, thereby not interfering with the complex formation and activation of the reductase by the iron protein. This is also the case when TN-NAD is the hydrogen acceptor instead of NAD (part **B** *).

Furthermore Table 1 demonstrates the strong inhibition of transhydrogenase activity by detergents (see l. c. [12, 13] for the mitochondrial transhydrogenase) which again does not influence the corresponding increase of activity by ferredoxin. The inhibition of the basal rate by heavy metals (e. g. 35% inhibition by 0.1 mM Zn^{++}; compare [14]) does not prevent activity increase by ferredoxin either.

p-chloromercuribenzoate, however, differs from the inhibitors tested, because it inhibits both the basal rate *and* the stimulation of activity.

The inhibitors listed so far affect either the basal activity and not the ferredoxin stimulated activity or both activities.

Reduced TN-NADP seems to inhibit in a third manner, as shown in Table 2. It does not inhibit the basal rate in the NADPH; NAD-system (column a) but inhibits the *additional* activity increment caused by ferredoxin (columns b to d). Therefore the ratio: total rate (+)Fd divided by the rate (–)Fd decreases with increasing inhibitor concentration (column e). As shown further, high concentration of ferredoxin has little competitive effect vs. the reduced analog.

In Fig. 1 the influence of TN-NADPH and NaCl on diaphorase activity with DCIP as hydrogen acceptor is shown. An part **A** the decrease of the *additional* activity caused by ferredoxin is plotted vs. ferredoxin concentration. In accordance with the findings in the transhydrogenase assay of Table 2 the iron protein is a poor competitor against the inhibitor. Some counteraction by Fd of TN-NADPH inhibition can be measured because in this particular diaphorase assay much more Fd is needed for good stimulation than in the transhydrogenase test (comp. l. c. [1]).

NaCl on the other hand inhibitis competitively with ferredoxin. The affinity constant of NaCl to the enzyme is at least 10^4-times greater than that of

No.	TN-NADPH	Rates expressed as $\Delta E_{340} \times 10^{-3}/10$ sec a (–) Fd	b (+) Ferredoxin 0.004 μM	c (+) Ferredoxin 0.04 μM	d (+) Ferredoxin 1.5 μM	e total activity (+) Fd / activity (–) Fd
	[mM]					
1	–	7.0	9.0 (0%)	13 (0%)	15 (0%)	3.1
2	0.025	7.0	5.0 (44%)	9.0 (31%)	10.3 (31%)	2.5
3	0.050	7.5	2.0 (78%)	3.6 (72%)	4.5 (70%)	1.6

Table 2. Inhibition of ferredoxin stimulated transhydrogenase activity by TN-NADPH: Influence of ferredoxin concentration. Data are mean values of 4 experiments. Those in columns b to d (= with ferredoxin added) are rates, from which the rates of column a (= without ferredoxin) are subtracted, thereby representing that part of activity only caused by ferredoxin. Data in brackets represent % inhibition of control (= data in line 1; compare also legend of Fig. 1). Column e shows the ratio of *total* activity with ferredoxin (1.5 μM) divided by activity without ferredoxin (= data in a); see also l c. [2], Fig. 4. NADP 0.1 mM; Fd-NADP reductase approx. 1.6 μM. In addition the reaction mixture contains the first 6 components mentioned in the legend of Table 1.

* This transhydrogenation can be done without a NADPH generating (isocitrate + isocitrate dehydrogenase) system, and gives identical rates. It is the most obvious and simple proof that the generating system used otherwise is not involved in the reactions at the reductase dealt with herein.

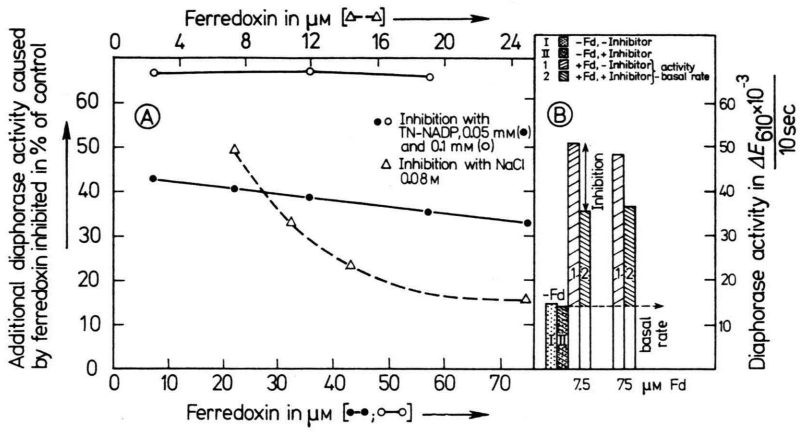

Fig. 1. Inhibition of ferredoxin stimulated diaphorase activity (with DCIP) by TN-NADPH and NaCl: influence of ferredoxin concentration. NADPH (0.1 mM) is regenerated by isocitrate; DCIP 0.1 mM; further components of the reaction mixture are the first 5 in the legend of Table 1; see also l. c. [1]. As in most of the other experiments TN-NADP is reduced by the transhydrogenase reaction in the same reaction mixture before the diaphorase reaction is started (see also Methods). In *A* percent inhibition is plotted of the ferredoxin stimulated part of activity (that is *total* activity with Fd minus activity without Fd) in the presence of inhibitors indicated. In *B* this calculation (of *A*) is illustrated for 2 Fd-concentrations with 0.05 mM TN-NADPH ●—●). Controls (= zero % inhibition) are columns No. 1.

TN-NADPH as may be roughly calculated from the percent inhibition data.

Part **B** of Fig. 1 explains and extends the data of part **A**. The basal rate is only slightly influenced by TN-NADPH (columns I, II). Inhibitions is therefore due to inhibition of the *additional* activity only, which is caused by ferredoxin. Only when inhibitor concentrations are more than 0.1 mM some percent inhibition of the rate without ferredoxin is observed.

Ferredoxin stimulated diaphorase activity (with DCIP) is also inhibited by high NADPH concentrations (>0.2 mM; the K_m is approx. 0.01 mM for NADPH), whereas the basal rate is not influenced by concentrations up to 6 mM. It takes about 0.7 mM for 50%, and 2 mM NADPH for a 75% inhibition of the activity increment which is observed in the presence of ferredoxin. For the latter decrease of activity only 0.05 mM TN-NADPH is needed. – These high NADPH concentrations are not very convincing in enzymatic investigations for reasons of side effects (e. g. a strong nonenzymic rate has to be taken into acount), but the data match those made with TN-NADPH.

Table 3 demonstrates the influence of TN-NADPH on two other diaphorase systems, namely oxygen uptake mediated by methylviologen and by ferredoxin or a mixture of both. The rate with methylviologen/O_2 (column 1) decrease somewhat with increasing inhibitor concentrations. Surprisingly, oxygen uptake mediated by *ferredoxin* is not affected at all (columns 3, 4). A mixture of both diaphorase reagents yields a rate which is higher

		O_2-uptake in µmoles × 10^{-2}/ml × min					
		(1)	(2)	(3)	(4)	(5)	(6)
Additions	[mM]	Methylviologen 0.5 mM	0.1 mM	Ferredoxin 4.2 µM	0.42 µM	Methylviologen 0.10 mM + ferredoxin 0.42 µM	% increase of activity
minus inhibitor		6.0	2.6	2.4	0.2	4.5/4.3	63
TN-NADPH	0.025	—	2.5	2.5	—	—	—
TN-NADPH	0.050	4.8	2.3	2.4	0.2	3.0/2.5	10
TN-NADPH	0.10	3.2	2.0	2.2	—	2.7/2.0	5

Table 3. Influence of TN-NADPH on oxygen uptake in diaphorase reaction systems. Column (5): mean values of 2 sets of experiments; column (6) shows the increase of activity measured in system (5) vs. the sum of activities of column (2) and (4). Ferredoxin-NADP reductase 0.46 µM; NADPH, 0.2 mM, is constantly regenerated by isocitrate. The assay contains in µmoles per 2 ml: TRIS-HCl, pH 7.0, 160; $MgCl_2$ 4; isocitrate 6; isocitrate dehydrogenase 0.38 mg/2 mls. Other compounds as indicated.

than the sum of their single rates (see first line) as was published in detail [1]. This *additional* O_2-uptake, due to the stimulatory effect of ferredoxin, is also inhibited by TN-NADPH (column 5).

Photosynthetic NADP reduction requires an undisturbed interaction of ferredoxin with the reductase [2, 3, 5, 6]. Under conditions which do not allow proper complex formation between the two proteins no NADP reduction can be observed. Fig. 2 presents data of photosynthetic reduction of NADP and TN-NADP. With *reduced* TN-NADP in the assay a strong decrease of the reduction rate occurs (see legend of Fig. 2; there is a technical difficulty regarding the exact determination of the concentration of TN-NADPH present in the moment when the readings are taken, because the Fd-NADP reductase of the chloroplast material causes a transhydrogenation from the (prereduced) TN-NADPH to NADP; see Table 6).

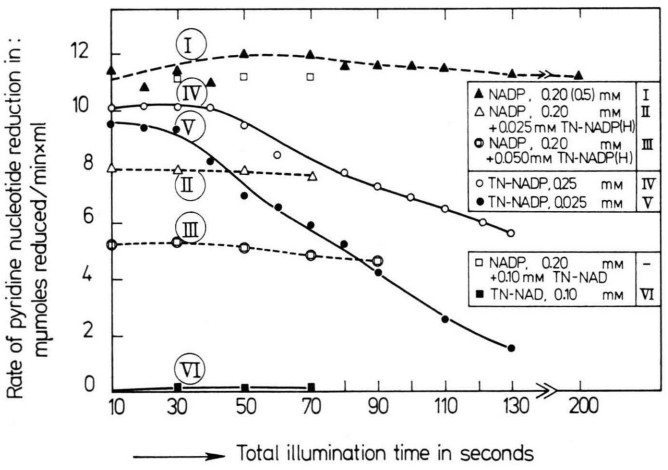

Fig. 2. Inhibition of photosynthetic NADP and TN-NADP reduction by reduced TN-NADP. 1 ml reaction mixture contains in µmoles: TRIS-HCl, pH 7, 60; $MgCl_2$ 2; inorganic phosphate 1; ADP 1.2; sucrose 60; ferredoxin 1.4×10^{-3}; Chl 13 µg/ml and the pyridine nucleotides as indicated in the inset. Chloroplasts are from 10 days old cultures of *Bumilleriopsis*. For inhibition experiments TN-NADP is photosynthetically reduced beforehand in the same reaction mixture (about 10 to 13 nmoles reduced during 100 to 120 sec (pre) illumination time). Then NADP is added, the mixture kept 1 min in darkness, then again illuminated and readings at 340 nm taken every 5 to 10 sec. The same results are obtained, when TN-NADP is reduced before separately by isocitrate (see Methods).

The degree of inhibition of NADP reduction is related to inhibitor concentration (curves II, III). Reduced NADP, however, formed during this photosynthetic reaction does hardly affect the reduction rate (curve I; after 200 sec of illumination time approx. 0.04 mM NADPH is formed. The rate of reduction is the same when using either 0.2 or 0.05 mM NADP). It was shown previously [2] that reduced NADP is generally a competitive inhibitor against its oxidized form at the same nucleotide binding site of the reductase. In this experiment the NADPH accumulating during the reaction is too small to compete with the concentration of NADP present.

Oxidized NADP and its sulfur analog are very similar with regard to the nucleotide binding site of the enzyme [2]. This is also indicated by almost the same rates obtained with NADP or TN-NADP during the first seconds of their photosynthetic reduction (curves I, IV). Therefore the inhibition of photosynthetic pyridine nucleotide reduction by TN-NADPH (with comparatively high concentrations of NADP present) was not expected.

One has to conclude that this inhibition is due to binding of this analog at a site different from the pyridine nucleotide binding site at the reductase; it is inferred that the analog interferes with reductase/ferredoxin interaction.

When TN-NADP serves as hydrogen acceptor the inhibition of its photosynthetic reduction occurs after 30 to 50 sec of illumination (curves IV, V) due to the formation of reduced TN-NADP. In curve IV competition of TN-NADPH with TN-NADP is negligible since there is 0.25 mM of the latter which equals NADP concentration in curve I, where no competition of NADPH vs. NADP is observed either. Consequently, inhibition by TN-NADPH shown in curve IV is of the same type as in curves II and III (with NADP as the hydrogen acceptor).

This is also evident in curve V, where TN-NADP is only 0.025 mM. Here a stronger inhibition is observed than in curve IV with 0.25 mM TN-NADP. Since in both experiments the reduction rate of TN-NADP is about the same, the ratio of TN-NADPH (formed) to TN-NADP (present) is much greater in curve V than in IV. Only in case of curve V, therefore, an *additional* inhibition due to a *competition* of TN-NADPH vs. its oxidized form for their pyridine nucleotide binding site is effective.

TN-NAD is not reduced by illuminated chloroplasts under the conditions given (curve VI) and does not inhibit photosynthetic reduction of NADP or TN-NADP (see square signs in Fig. 2).

Inhibition by TN-NADPH of photosynthetic NADP reduction is almost independent of NADP concentration (Table 4). This is also measured when TN-NADP replaces NADP as hydrogen acceptor. Increasing concentrations of ferredoxin relieve the inhibition somewhat, but the effect is small. This is similar to the findings in the other assay systems described herein.

Rates in $\Delta E_{340} \times 10^{-3}$/10 sec	(−) TN-NADP	(+) TN-NADP	% Inhibition
A. influence of NADP			
NADP, mM			
0.2	9.8	5.0	49
0.8	12.1	6.5	46
1.2	11.6	6.7	42
B. influence of ferredoxin			
Fd, μM			
1.45	15.0	6.8	53
2.90	20.0	10.0	50
3.60	21.0	11.0	48
11.0	18.8	11.4	40

Table 4. Inhibition of photosynthetic NADP reduction by TN-NADPH: influence of NADP (A) and ferredoxin (B). Reaction mixture and method see legend of Fig. 2; TN-NADP(H) 0.05 mM. Data are from 3 experiments; to A: ferredoxin 0.35 μM; Chl 21 μg/ml; to B: NADP 0.2 mM; Chl approx. 10 μg/ml.

No inhibition by reduced TN-NADP is observed during photosynthetic reduction of mammalian cytochrome c (Table 5) up to 0.026 mM inhibitor concentration. Concentrations above that figure increase the dark rate, which masks the photosynthetic (light) rate. A decrease of activity is never seen, instead the rate − corrected for the dark reaction − is slightly increased.

The equilibrium constant $K = \frac{\text{NADP} \times \text{TN-NADPH}}{\text{NADPH} \times \text{TN-NADP}}$

Rates in $\Delta E_{550} \times 10^{-3}$/10 sec	(−) TN-NADPH	(+) TN-NADPH	
Ferredoxin		0.014 mM	0.026 mM
0	7.0	—	7.5
0.38 μM	14.0	19.0 (3)	21.0 (5)
0.76 μM	19.5	25.0 (4)	27.0 (6)

Table 5. Photosynthetic reduction of mammalian cytochrome c: influence of reduced TN-NADP. Reaction mixture see legend of Fig. 2; Chl 3.7 μg/ml; cytochrome c 1.25 mg/ml. TN-NADPH is given reduced beforehand. The rates of cytochrome reduction in the dark are in brackets and have to be subtracted from the rates given to obtain the values due to the light driven reaction. Chloroplast material is from a thin, 2 days old culture of *Bumilleriopsis filiformis*.

is close to unity as can be calculated from columns a/3 and c/3 or a/2 and b/2 of Table 6. This implies the TN-NADP/TN-NADPH couple has about the same (or a little more positive) standard redox potential as the parent nucleotide (compare l. c. [15]). It should be pointed out that the equilibrium is freely reversible, which can be proved by using TN-NADPH as hydrogen donor to start the reaction and NADP as H-acceptor.

An adequate amount of chloroplast material (from *Bumilleriopsis* and active in photosynthetic electron transport in vitro) can be substituted for the purified reductase. By doing so, neither ATP (0.1 to 1 mM), nor some minutes of illumination with white light, with or without cofactors added for optimal electron transport (like phenazinium methosulfate or K-ferricyanide, see l. c. [8]) will shift this equilibrium. No transhydrogenation is observed under these conditions when NADH is used as hydrogen donor instead of NADPH. Hence, there is no indication of an energized transhydrogenase reaction in chloroplast particles as described by LEE and ERNSTER [16] for mitochondrial vesicles or for photosynthetically active chromatophores of *Rhodospirillum rubrum* [17].

	(a) in micromolar concentration	(b)		(c)	
	NADPH given	TN-NADPH formed		TN-NADPH formed	
		(with 50 μM TN-NADP)	NADPH remained / TN-NADPH formed	(with 100 μM TN-NADP)	NADPH remained / TN-NADPH formed
1.	28	16	0.75	25	0.12
2.	55	27	1.03	40	0.38
3.	110	28	2.90	53	1.07

Table 6. Equilibrium of the transhydrogenation reaction: NADPH + TN-NADP ⇄ NADP + TN-NADPH. Equilibrium is measured by optical densities at 340 and 400 nm, respectively, in 0.08 M TRIS-HCl, pH 7.0. Transhydrogenase (= Fd-NADP reductase) is 0.3 μM. Equilibrium is attained within some seconds. The concentration of transhydrogenase used can be substituted by chloroplast material from *Bumilleriopsis* equivalent to 10 to 15 μg of chlorophyll/ml.

Discussion

In previous papers [1–3] it was shown that Fd-NADP reductase has one specific site for ferredoxin and one for pyridine nucleotides whether these are oxidized or reduced. Transhydrogenase and diaphorase activities therefore can be competitively inhibited by (oxidized) pyridine nucleotides (or 2′-AMP) although the percent stimulation by Fd of the inhibited rates is not changed (Table 1 and Fig. 4 in l. c. [2]).

Reduced TN-NADP deviates from this rule since it abolishes the stimulatory effect of ferredoxin on these enzymic activities. Photosynthetic NADP or TN-NADP reduction is also inhibited by it (Fig. 2). This inhibition is noncompetitive to NADP (or TN-NADP, see Table 4); the extent of inhibition is roughly in the same order in all three activities which are due to the presence of ferredoxin. – It appears therefore that the inhibitions is not related to the pyridine nucleotide binding site of the reductase

It is suggested that **a.**) there is the same type of complex formation between the reductase and ferredoxin which causes all three ferredoxin stimulated enzymic activities dealt with herein, and **b.**) this inhibitor changes the mode of the reductase/ferredoxin interaction.

This is further corroborated by the finding that photosynthetic cytochrome c reduction is not influenced by TN-NADPH (Table 5). This reduction does not need the presence of ferredoxin-NADP reductase [18, 8]. Consequently the inhibitor does not exert its effects on the ferredoxin molecule itself.

The inhibition resembles in part the action of sodium pyrophosphate, NaCl and $MgCl_2$ (see introduction). These salts inhibit transhydrogenase activity stimulation by ferredoxin or oxygen uptake mediated by ferredoxin. It was shown that they destroy the reductase/ferredoxin complex so that the effects due to this complex cannot be observed [1, 3].

Pyrophosphate reacts with the reductase and not with ferredoxin [6]. Present in high and unphysiological concentrations the salts mentioned above react rather unspecifically with both the pyridine nucleotide and the ferredoxin binding site. Therefore some inhibition of the basal rate (= rate with no ferredoxin present in the assay) of e. g. transhydrogenase activity is generally observed (see Fig. 6 in l. c. [1]). FOUST et al. [19] also noticed that ionic strength influenced the binding of both NADP and ferredoxin to the reductase (from spinach). In addition, inactivation of the reductase itself may occur (see l. c. [20]). The salt inhibition therefore can be counteracted by higher Fd concentrations on the one hand (see Fig. 1, △ - - - △ for NaCl; l. c. [5] for pyrophosphate) and with higher pyridine nucleotide concentrations on the other [7].

The inhibition by TN-NADPH of ferredoxin stimulated enzymic activity increments is noncompetitive vs. ferredoxin. Together with the lack of inhibition of ferredoxin mediated oxygen uptake, it is suggested that the particular binding site of reduced TN-NADP responsible for the noncompetitive action is not identical with that for ferredoxin. This is further evidenced by the DCIP-diaphorase reaction which is not influenced by the inhibitor, whereas the methylviologen mediated oxygen uptake is inhibited to a certain extent. That is to say, the inhibitor appears to bind at the reductase at a third site different from that one for ferredoxin and the one for pyridine nucleotides. Three binding sites were proposed by CHUNG [21] for the transhydrogenase from Azetobacter (see also l. c. [22]).

TN-NADPH may bind close to the site where the diaphorase substrates bind or react with the enzyme that some of them (e. g. methylviologen) may be (sterically) hindered from proper enzyme binding or reaction with the FAD moiety, others – like DCIP – are not. In addition ferredoxin does not compete with TN-NADPH, the oxygen uptake via ferredoxin is not affected. Conclusively, the reductase can still react with ferredoxin even in the presence of the nucleotide analog. TN-NADPH, however, abolishes the stimulatory effect of ferredoxin on the activities of the enzyme. This specific mode of action is in definite contrast to pyrophosphate inhibition, which does not allow reductase/ferredoxin interaction at all.

It is tempting to suggest tha TN-NADPH inhibits the activation of the reductase by its (positive) effector ferredoxin. Hence, the (additional) role of ferredoxin as an enzyme effector already assumed from previous studies [1–3] appears to be substantiated by these inhibitor experiments.

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Experimente zur intramolekularen Energieleitung in BU-DNA des Phagen PBSH aus *Bacillus subtilis* nach Bestrahlung mit langwelligem UV

Experiments Concerning the Intramolecular Energy Transfer in BU-DNA of the Phage PBSH from *B. subtilis* after Long-wave UV-irradiation

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The production of BU-phages with a relative high amount of hybrid DNA is described. Transforming experiments with the irradiated phage BU-DNA and the investigation of the photodecomposition of the single strands of hybrid DNA demonstrate clearly intramolecular energy transfer. From the breakage rates of the single strands of hybrid DNA a calculation for the relative amount of intramolecular energy transfer in hybrid BU-phage DNA is given.

I. Einleitung

In der vorangehenden Arbeit [1] wurde gezeigt, daß die Bestrahlung von hybrider bakterieller TB-DNA [2] mit langwelligem UV-Licht ($\lambda \approx 313$ nm) nicht nur eine Schädigung des B-Stranges, sondern auch des T-Stranges hervorruft. Dies erscheint bemerkenswert, weil langwelliges UV-Licht nur BU-haltige DNA schädigt [1]. Wir bezeichneten diesen Prozeß der Energieübertragung vom B-Strang zum T-Strang in der hybriden DNA als „intramolekulare Energieleitung". Diese intramolekulare Energieleitung äußerte sich einmal in einem stärkeren Abfall der Transformationsrate von TB-DNA nach Bestrahlung als erwartet (biologischer Test), zum anderen im Auftreten von Einzelstrangbrüchen im T-Strang hybrider DNA nach Bestrahlung. Dies wurde im Dichtegradienten einer analytischen Ultrazentrifuge (AUZ) nachgewiesen (physikalischer Test).

Diese Resultate sollten nun an einem neuen System entweder erhärtet oder in Frage gestellt werden. Weiterhin bringt die Auswertung der Bruchraten von bakterieller DNA in der AUZ infolge der Molekulargewichtsinhomogenitäten einige Schwierigkeiten mit sich [1]. Um diese Mängel zu beseitigen, suchten wir nach einem neuen System mit DNA von

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