Relationship between Uridine Nucleotide Sugar Activation of Glutamic Dehydrogenases in Fungi and Existence of Chitin and Cellulose in Their Walls.

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Received December 23, 1970

Uridylates, uridine nucleotide sugars and uridine nucleotide amino sugars function as allosteric activators of DPN-linked glutamic dehydrogenases of some fungi. The effect appears to be restricted to glutamic dehydrogenases obtained from those 'cellulosic' fungi that do not synthesize chitin in their cell walls. These glutamic dehydrogenases have also retained the ability to interact with five other activators that had been found for all members of the Oomycetes (LéJohn, Stevenson and Meuser, *J. Biol. Chem. 245.* 5569, 1970).

Vogel (1) has shown that the lower fungi can be separated into two distinct categories based on the pathway used for biosynthesis of lysine. Hypochytridiomycetes and Oomycetes synthesize lysine via the α,ε-diaminopimelic acid (DAP) pathway while the Chytridiomycetes and Zygomycetes, like all higher fungi, utilize the α-aminoadipic acid (AAA) pathway. Hutter and DeMoss (2) observed that the distribution of these two biosynthetic pathways parallels the separation of chitin and cellulose in the cell walls of the lower fungi and relates to the organization of enzymes of the tryptophan biosynthetic complex. Recently, we showed that a further correlation exists at the level of enzyme controls (3, 4). Different families of allosteric modifiers modulate the DPN-linked glutamic dehydrogenases commonly found in all the lower fungi. No direct connection between lysine biosynthetic paths, cell wall structure and enzyme regulation (organization) could be observed (4). However, a new set of allosteric modulators have been discovered which could provide fresh clues that may link one of the multivalent controls of the glutamic dehydrogenases with cell wall structure. Uridylates, and UDP-compounds (hexoses, pentoses, and amino sugars), specifically, activate the enzymes obtained from all fungi that are known to be devoid of chitin in their cell walls.

Pyrimidine nucleotides, hitherto, had never been observed to affect glutamic dehydrogenases from any source (5).

MATERIALS AND METHODS

DPN-linked glutamic dehydrogenases were purified from *Pythium debaryanum*, *P. splendens*, (Peronosporales), *Achyla sp.*, *Saprolegnia ferax*, *Thraustotheca clavata*, (Saprolegniales) and *Rhizidiomyces apophysatus* (Hypochytridiales) as described previously (6). The enzyme used from all other organisms listed in the footnote to Table I were partially fractionated cell-free extracts. The protein that precipitated between 45% and 60% with ammonium sulfate from the supernatant of extracts treated with 2% protamine sulfate solution (1 part protamine sulfate : 3 parts cell extract) was used as the source of enzyme. This preparation was desalted on Sephadex G-25 column before use in most cases. The method used for the purification of glutamic dehydrogenases from *Blastocladiella emersonii* and *Allomyces arbuscula* (Blastocladiales) has been described (8, 9). The other Phycomycetes used in this study were *Rhizophlyctis rosea*, *Entophlyctis* sp. (Chytridiales) and *Mucor hiemalis*, *Rhizopus stolonifer* (Mucorales). The enzymes from these organisms were obtained from material precipitating between 50% and 60% with ammonium sulfate treatment.

Chemicals were obtained from Sigma Chemical Co. and P-L Biochemicals.

RESULTS AND DISCUSSION

Current knowledge about the major carbohydrate polymers in the cell walls of the Phycomycetes is summarized in Table I (see ref. 7). The known pathways for biosynthesis of lysine that is used by these fungi is also recorded in the Table. All of the Phycomycetes possess only the type of glutamic dehydrogenase that is DPN-linked (3). The glutamic dehydrogenases obtained from several Orders of the Oomycetes and Hypochytridiomycetes have been shown to be activated by metabolites such as TPN^+, P-enolpyruvate, guanylates, short chain acyl CoA derivatives, and either ATP or AMP (3). This analysis has now been extended to include members of the Leptomitales and similar results were obtained (Table I). However, a sixth class of activators was recently discovered for

Table I. *Distribution of the multivalent activating effects of pyridine nucleotides, purine nucleotides, short chain acyl CoA derivatives, P-enolpyruvate and UDP-compounds among DPN-linked glutamic dehydrogenases of the lower fungi.*

Organism	Major Wall Component	Lysine Path	Activator (Groups) PEP	TPN[a]	GTP[b]	CoA[c]	UTP[d]
Oomycetes							
Saprolegniales	'Cellulose'	DAP	+	+	+	+	+
Leptomitales*	'Cellulose' + Chitin	DAP	+	+	+	+	0
Peronosporales	'Cellulose'	DAP	+	+	+	+	+
Hypochytridiomycetes							
Hypochytridales*	Cellulose + Chitin	DAP	+	+	+	+	0
Chytridiomycetes							
Chytridiales	Chitin	AAA	0	0	0	0	0
Blastocladiales	Chitin	AAA	0	0	0	0	0
Zygomycetes							
Mucorales	Chitin	AAA	0	0	0	0	0

(+) signifies activation and (0) no effect.
*Chitin content confirmed by X-ray analyses in at least one species (see ref. 11, 12).

(a), TPN^+; TPNH, deamino-TPN^+; (b), GTP, GDP, GMP, ATP; (c), short chain acyl CoA derivatives; (d), uridine nucleotides, uridine nucleotide sugars, uridine nucleotide amino sugars.

Organisms tested. Saprolegniales (*Achlya sp* 1969; *A. flagellata; Saprolegnia sp; S. ferax; Thraustotheca sp; T. clavata; Isoachlya itoana* and *Aphanomyces euteiches*).
Leptomitales (*Leptomitus sp; Apodachlya sp* 47-17; *Sapromyces androgynus* and *Mindeniella sp.*).
Peronosporales (*Pythium debaryanum; P. butleri; P. splendens* and *P. cantenulatum*.
Hypochytridiales (*Rhizidiomyces apophysatus* and *Hypochytrium catenoides*).

glutamic dehydrogenases of the Oomycetes. These are uridine nucleotides and UDP-nucleotide sugars. The uridine compounds show some specificity toward the various glutamic dehydrogenases. As shown in Table I, the enzyme from the Saprolegniales and Peronosporales are activated by UDP-derivatives but those from the Leptomitales and Hypochytridiales are not.

A summary is given, in Table I, of the distribution of uridine nucelotide activation of the glutamic dehydrogenases among 19 species of the Oomycetes and Hypochytridiomycetes studied. All members of the Saprolegniales tested have an enzyme that is

activated. Without exception, the glutamic dehydrogenases from the Leptomitales were unaffected by uridine nucleotides. On the other hand, activation was quite pronounced on the enzymes obtained from all of the Peronosporales. Consequently, the analytical and kinetic studies were carried out on purified glutamic dehydrogenases from the Peronosporales.

A survey was made of the influence of several nucleotide sugars known to be involved in the biosynthesis of cell walls in different organisms (10). The results are presented in Table II. Although guanylates activate the glutamic dehydrogenases markedly, none of the GDP nucleotide sugars had any effect. Most of the UDP nucleotide sugars and amino sugars activated the glutamic dehydrogenase from *Pythium debaryanum* and *P. splendens*. Interestingly, UDP-galactose did not activate significantly.

Other pyrimidine and purine nucleotides (CTP, CDP, CMP, ITP, IDP, IMP, TTP, TDP, TMP and the deoxynucleotides of cytidine) had no effect. These have not been included in the Table. Amino sugars were also ineffective. The specificity for the UDP moiety in Pyrimidine nucleotide activation is therefore quite evident.

Table II. *Activation of DPN-linked glutamic dehydrogenase of Pythium splendens by uridine nucleotides (sugars and amino sugars), coenzymes and other metabolites.*

Assay system consisted of 10 mM α-ketoglutarate, 0.167 mM DPNH, 50 mM NH_4^+, 67 mM Tris-acetate, pH 8 and 3 μg of enzyme in the reductive amination reaction. The oxidative deamination reaction is considerably less activated by these compounds.

Addition	mM	v_e/v_o ‡	Addition	mM	v_e/v_o
Nil	-	1.0	TPN^+	0.166	9.6
UTP	0.33	5.1	Deamino-TPN^+	0.166	9.0
UDP	0.33	5.0	Acetyl CoA	0.33	5.1
UDP-glucose	0.33	4.8	GTP	0.33	6.7
UDP-N-acetyl glucosamine	0.33	4.2	P-enolpyruvate	0.50	10.0
UDP-xylose	0.33	4.0	ATP	1.0	1.5
UDP-mannose	0.33	5.5			
UDP-glucuronic acid	0.33	4.8			
UDP-galactose	0.33	1.2			
GDP-glucose	0.33	1.1			
GDP-mannose	0.33	1.3			

‡ v_e is reaction rate in the presence of effector and v_o is rate withou effector.

Kinetics. To compare the affinity of the glutamic dehydrogenases for the uridine nucleotides with determined affinities of all the other activators (3), rate-concentration activation studies were carried out and the results are shown in Fig. 1. Since the activators of these enzymes function optimally when the concentration of substrates is high, the procedure outlined in our recent communication (3) was followed to determine the activation constants

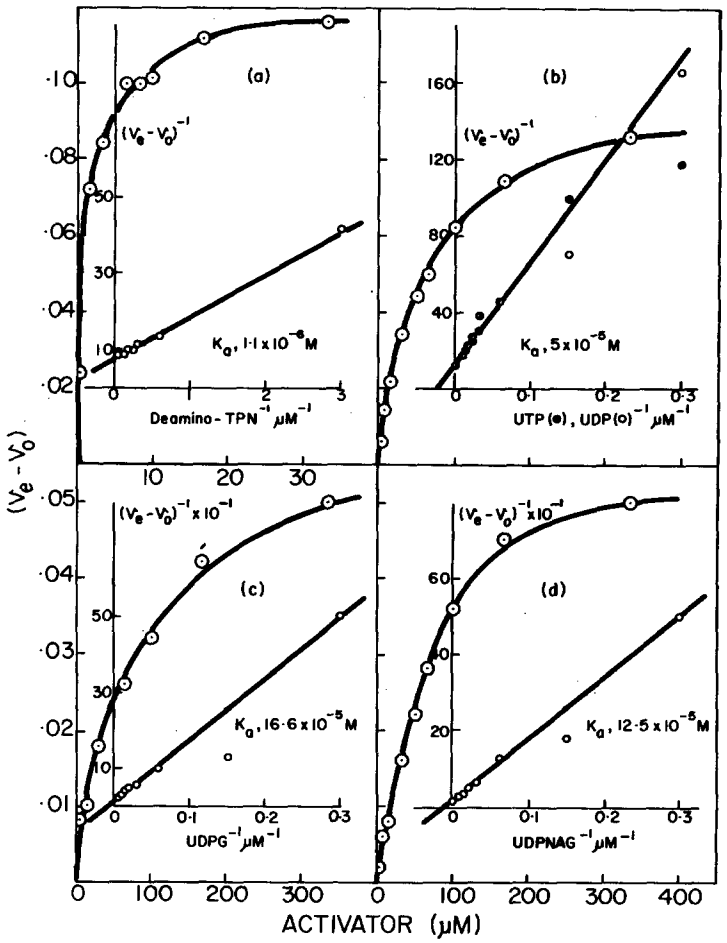

Fig. 1. *Determination of the activation constants of uridine nucleotides, uridine nucleotide sugars and TPN analog in their interaction with Pythium debaryanum DPN-linked glutamic dehydrogenase.* (a) Deamino-TPN (b) UTP and UDP (c) UDP-glucose (d) UDP-N-acetylglucosamine. Reaction system consisted of 0.166 mM DPNH, 10 mM α-ketoglutarate, 50 mM NH_4^+, 67 mM Tris-acetate, pH 8, 2 μg enzyme. Reaction rates in the presence (v_e) and in the absence (v_o) of effector.

of the modulators. The glutamic dehydrogenases had higher affinities for uridylates (Fig. 1b) than the nucleotide sugars and amino sugars (Fig. 1c and d). The activation constants varied from 50 μM to 150 μM (in units of concentration).

It was also found that the analog of TPN^+ (deamino-TPN^+) could serve, with almost equal efficiency, as an activator, as does TPN^+ (Fig. 1a). This analog is only 10% to 12% reactive with TPN-linked dehydrogenases present in these organisms. Those tested include TPN-linked isocitric, glucose-6-phosphate, and malic dehydrogenases. The activation constant for deamino-TPN^+ is 1.1 μM compared to 0.33 μM for TPN.

CONCLUSIONS

The allosteric response of the glutamic dehydrogenases of these fungi towards uridine nucleotides, UDP-sugars and amino sugars suggests that biosynthesis of UDP-nucleotide sugars may be intimately connected with glutamate biosynthesis. Glutamine is normally used as the amino-donor in biosynthesis of amino sugars. We have not been able to observe any activating effect of uridine nucleotides on the glutamine synthetases from these organisms. This is not too surprising since glutamic dehydrogenase is the first enzyme of the pathway. Conservation of the organic amino group may be the critical factor. However, if the uridine nucleotides do activate the glutamic dehydrogenases so as to provide glutamine for amino sugar biosynthesis, then a reasonable argument is that related organisms within this group, known to synthesize chitin in their walls, should have glutamic dehydrogenases that are insensitive to uridine nucleotides in contrast to those that do not. One would expect that the enzymes required for synthesis of amino sugars would be constitutive in those cells that can synthesize chitin easily. The results presented support this conclusion. *Rhizidiomyces apophysatus* and *Apodachlya* sp (47-17) (see Table I) have been shown by X-ray studies to contain cellulose and chitin in their walls (11, 12). Both organisms have uridine nucleotide-insensitive glutamic dehydrogenases. *P. butleri* (13) and *P. debaryanum* (14) which have been shown, convincingly, to be devoid of chitin and have only traces of hexosamines in their walls are extremely sensitive to activation by uridine nucleotides.

Notwithstanding the speculation on the possible physiological

reason for the activating effects of uridine nucleotide sugars on the glutamic dehydrogenases, the occurrence and specificity of this allosteric effect should provoke similar studies on higher plants and algae that are known to contain cellulose and no chitin in their walls.

ACKNOWLEDGEMENTS

This work was supported by a grant from the National Research Council of Canada. The help of Drs. R. Emerson (Berkeley), J.S. Lovett (Purdue), M. Fuller (Georgia), E. Beneke, A. Rogers (Michigan State) in providing me with some fungal cultures is appreciated.

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