Romanian Biotechnological Letters *Vol. 16, No. 2, 2011*
Copyright © 2011 University of Bucharest Printed in Romania. All rights reserved
SHORT COMUNICATION

Unexpected effect of some dinitrophenyl derivatives on yeast fermentation

Received for publication, October 30, 2010
Accepted, March 3, 2011

IOAN-MARIAN RIŞCA,[*1] ALIN IONAŞ,[**] IONEL MANGALAGIU[**]
[*] *Faculty of Silviculture, "Stefan cel Mare" University, 13 University str., RO-720229, Suceava, Romania, phone/fax 0230521664*
[**] *Faculty of Chemistry, "Al. I. Cuza" University, 11 Carol I, RO-700506 Iasi, Romania, phone/fax 004032201313*
[1] *Corresponding author: E-mail, risca@usv.ro*

Abstract

It is assumed that dinitrophenols hinder the proton translocation through the mitochondrial inner membrane, inhibiting thus the oxidative phosphorylation. Some other authors claim that proton translocation could be a secondary process, independent on ATP formation. Therefore, we have investigated the biological effect of several 2,4-dinitrophenyl derivatives on the fermentation produced by yeast strains. The biological effect of all these compounds depends on the chemical structure of the replacing radical of the phenolic protons. The dinitrophenyl ethers clearly stimulate the fermentative processes.

Keywords**:** dinitrophenyl derivatives, yeast fermentation, toxicity mechanisms.

Introduction

Dinitrophenols and their derivatives have multiple biological effects, being used in agriculture as fungicides, herbicides and insecticides [1] or in medicine and biology as metabolic inhibitors [2]. The fungicidal, herbicidal or insecticidal properties depend on minor differences in the chemical structure; the general chemical structure of dinitrophenol pesticides is presented in **Fig. 1**.

It was also assumed that dinitrophenols (DNP) reduce the proton translocation through the mitochondrial inner membrane and therefore oxidative phosphorylation is inhibited. The permeability of mitochondrial membranes to protons is increased with the failure of conversion of ADP to ATP [3-6].

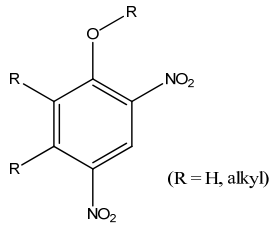


Fig. 1. General chemical structure of dinitrophenol pesticides.

Instead, the energy produced due to the oxidation elevates the body temperature which can lead to hyperpyrexia and the inefficiency of the circulatory and respiratory processes conduces to anoxia and acidosis. Because fat serves as an alternative source of energy, in case of exposure to dinitrophenol derivatives, weight loss occurs as a result of inhibition of lipogenesis from pyruvate and lactate [6, 7].

All these effects were associated with the presence of nitro groups in positions 2 and 4 of the benzene ring, and a free or potential free hydroxyl group. Some studies showed that free hydroxyl group might be less important than the absorption of electromagnetic radiation around 6000 cm^{-1} [8-10].

Some authors [10] hypothesized that the biological effect of dinitrophenols can be associated with the energy transfer from the respiratory chain to ATP-synthase and not with proton translocation as the chemiosmotic hypothesis claims [3-5].

Therefore, the present study was undertaken in order to investigate thoroughly the effects of some dinitrophenyl derivatives on yeast fermentation process. These category of experiments are easy, cheap, fast and

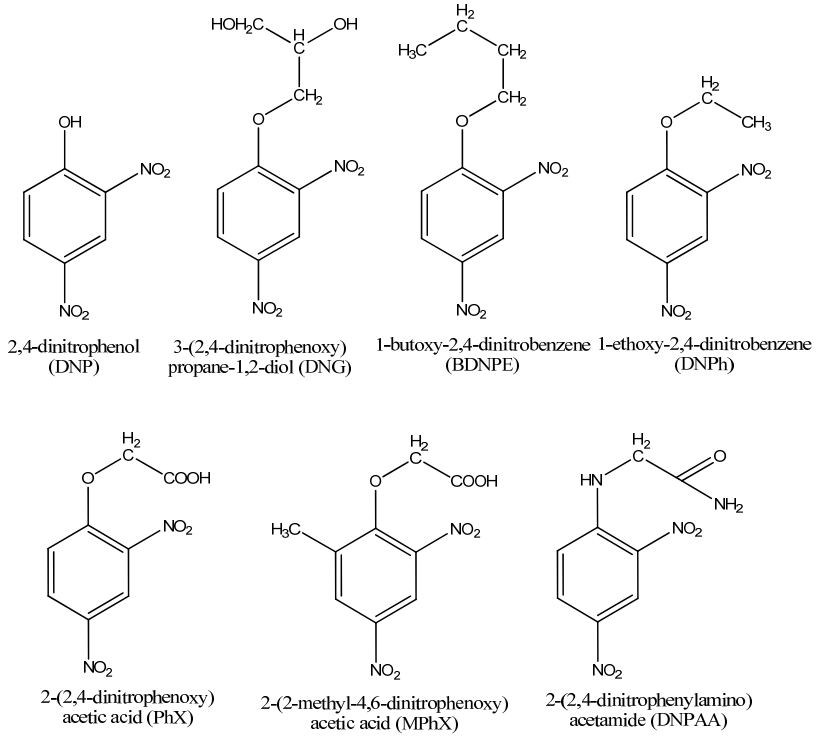
spectacular, they can reveal if these compounds act as ethers or phenols [11]. The possible mechanisms of toxicity of these chemicals and pesticides are discussed in the light of Eugen Macovschi's biostructural theory [11, 12] as well as of Peter Mitchell's chemiosmotic hypothesis [4, 5]**.**

Materials and Methods

Equipment. An orbital stirrer GFL 3005 and laboratory glassware were used.

Chemical reagents. All chemicals were of analytic purity (Merck, LabChema-Chemapol, Chinoin, Chimopar) and all solutions were prepared with redistilled water.

Treatment solutions. Several dinitrophenol ethers and dinitrophenols such as 2,4-dinitrophenol (2,4-DNF), 2,4-dinitroanisole (2,4-DNAN), and 3-(2,4-dinitrophenoxy)propane-1,2-diole) DNG, etc., were synthesized previously, starting from dinitrochlorobenzene, in the Biochemistry laboratory, Faculty of Chemistry, Al. I. Cuza University. Treatment solutions of dinitrophenyl ethers and dinitrophenols with the concentrations of 10^{-4} M were prepared. The following compounds were used in the study (**Fig. 2**):



2,4-dinitrophenol (**DNP**)
3-(2,4-dinitrophenoxy) propane-1,2-diol (**DNG**)
1-butoxy-2,4-dinitrobenzene (**BDNPE**)
1-ethoxy-2,4-dinitrobenzene (**DNPh**)
2-(2,4-dinitrophenoxy) acetic acid (**PhX**)
2-(2-methyl-4,6-dinitrophenoxy) acetic acid (**MPhX**)
2-(2,4-dinitrophenylamino) acetamide (**DNPAA**)

Fig. 2. Some dinitrophenol derivatives

Biological material. A yeast strain of *Saccharomyces cerevisiae* was used.

Procedure. Suspensions containing 10 g L^{-1} yeast, 50 g L^{-1} sucrose, 0.40 g L^{-1} KH_2PO_4 and the corresponding 10^{-4} M dinitrophenyl derivative were poured for fermenting – in three replicates – in 300 ml Erlenmeyer flasks, provided with a CO_2 capture system.

The fermentation processes lasted for 48 h, at 22 ºC, on an orbital stirrer (150 rot/min.). A control sample containing only yeast, sucrose and potassium phosphate salt was introduced.

The released carbon dioxide (CO_2) was captured in a NaOH 3 M solution. At the ending of the fermentation period, 300 ml $BaCl_2$ 20% solution and 2 drops of an alcoholic solution of 1% thymolphthalein were added in each flask. The NaOH excess was titrated with HCl 1.5 N, until the blue color disappeared [13].

The released CO_2 amount – in g CO_2/l solution – was calculated according to Hibbert and Gooding [14] as average value of the three repetitions, validated for a signification level of 95%.

Results and Discussion

Table 1 shows the effect of dinitrophenyl compounds on yeast fermentation in batch conditions. DNP proved to inhibit CO_2 production, whereas DNG and BDNPE induced an increase in the fermentation process. The dinitrophenol inhibition of the fermentation can be related to its relatively high concentration. In general, dinitrophenols stimulate the fermentation and inhibit the respiratory processes. Probably, dinitrophenyl ethers need a higher concentration to prove their toxic effect. At lower concentration all the dinitrophenyl ethers used here proved to have a significant (P = 5%) stimulatory effect on yeast fermentation. As expected, DNPAA, which does not contain the dinitrophenoxy moiety, had no effect on yeast fermentation. When the phenolic oxygen is replaced by another atom, such as the amine nitrogen, the biological effects are insignificant, such as in the case of the 2-(2,4-dinitrophenylamino)acetamide.

Table 1. CO_2 amount produced by yeast fermentation in the presence of various dinitrophenyl derivatives and related compounds

Dinitrophenol derivatives	CO_2 - average values (g L^{-1})
Control	**22.064 ± 0.799**
DNP	**20.325 ± 0.185**
DNG	**26.141 ± 0.398**
BDNPE	**25.222 ± 0.421**
DNPh	**22.348 ± 1.295**
PhX	**23.027 ± 0.478**
MPhX	**23.216 ± 0.319**
DNPAA	**22.127 ± 0.923**

By the replacement of the phenolic protons with various radicals and the formation of ethers of the 2,4-dinitrophenol, the inhibitory effect disappears – as in the case of ethyl (DNPh) or acetyl radicals (PhX si MPhX). Apparently, the mobile hydrogen atoms could have an inhibitory effect on yeast fermentation. However, carbon dioxide may appear both in the respiration and fermentation, which complicate the mechanism of dinitrophenol activity. In addition, dinitroanisole (DNPh) had no effect on yeast suspensions. Previous research showed that yeast cannot replace the OCH_3 group with an OH one [10]. Therefore, we suspect that the concentration of the investigated compounds play a key role in toxicity. Further investigation is necessary to establish the complex mechanism of dinitrophenyl derivatives investigated here. For example, oxygen consumption may be as important as carbon dioxide production in understanding the effect of dinitrophenols.

It is also important to note that the toxicity mechanism cannot be as simple as the chemiosmotic outlook claims, and a certain effect of dinitrophenyl compounds on the biostructure of yeast might be accepted.

However, our simple experiment suggests that some dinitrophenyl ethers increase carbon dioxide production of yeast suspensions. These results could not be *a priori* known since the replacing the OH groups in dinitrophenol molecules with OR, in which R is an alkyl group, have to cancel the uncoupling effect.

Conclusion

Dinitrophenols and the dinitrophenyl ethers used in this experiment showed a measurable effect on yeast fermentation in the presence of sugar. DNG and DNP had a different behavior under the experimental conditions, probably because of their molecular structures. DNPh and DNPAA had no effect on yeast fermentation. Further research is needed to understand the mechanism of toxicity of dinitrophenyl derivatives and their effect on yeast fermentation.

Acknowledgments

The authors are grateful to G. Drochioiu and C. A. Dumitras-Hutanu for the synthesis of some dinitrophenyl derivatives used in this study and for their suggestions. The financial support of this work by MedCT-ANCS, CNMP 2746/2007 (Grant 31-017/2007) is gratefully acknowledged.

References

1. D. JANZ, *Dinitrophenols.* In *Encyclopedia of Toxicology.* 2nd Ed., Vol. 2., P. Wexler (ed.), Academic Press, San Diego, 59-60 (2005).
2. E. COLMAN, Dinitrophenol and obesity: An early twentieth-century regulatory dilemma, *Regulatory Toxicol. and Pharmacol.*, **48**, 115–117 (2007).
3. P. MITCHELL, *Nature*, **191**, 144-148 (1961).
4. P. MITCHELL, *Chemiosmotic Coupling and Energy Transduction*, Glynn Research, England (1968).
5. P. MITCHELL, *David Keilin's respiratory chain concept and its chemiosmotic consequences*. Nobel Lecture, 1978.
6. R. M. HOLLINGWORTH, *Inhibitors and uncouplers of mitochondrial oxidative phosphorylation,* In *Handbook of Pesticide Toxicology.* 2nd Ed., Vol. 2. R. Krieger (ed.), Academic Press, San Diego, 1169-1261 (2001).
7. J. E. CASIDA, *Pest toxicology: The primary mechanisms of pesticide action.* In *Hayes' Handbook of Pesticide Toxicology.* 3rd Ed., Vol. 1., R. Krieger (ed.), Academic Press, San Diego, 103-117 (2010).
8. R. MOLNAR, D. CREANGA, R. RUSCANU, M. MURARIU, C. CIOBANU, M. I. NEICA, A. PUI, G. DROCHIOIU, *Lucr. Stiint. USAMV Iasi, s. Agric*., **49**, 352-357 (2006).
9. G. DROCHIOIU, Laser-induced ATP formation: Mechanism and consequences. *Photomed. Laser Surg*., **28**, 573-574 (2010).
10. C. A. DUMITRAS-HUTANU, A. PUI, Ş. JURCOANE, E. RUSU, G. DROCHIOIU, Biological effect and the toxicity mechanisms of some dinitrophenyl ethers. *Rom. Biotechnol. Lett*. **14**(6), 4893-4899 (2009).
11. G. DROCHIOIU, C. ONISCU, R. GRADINARU, M. MURARIU, The biostructural theory versus the chemiosmotic theory. *Roum. Biotechnol. Lett*., **9**(2), 1579-1586 (2004).
12. G. DROCHIOIU, *Eugen Macovschi's concept of biostructure and its current development*. In *Life and mind. In search of the physical basis*.,S. Savva (ed.) Trafford Publ., Canada, USA, Ireland & UK, 43-60 (2006).
13. G. STEFANIC, Determinarea nivelului potenţial al respiraţiei solurilor în condiţiile întreţinerii concentraţiei oxigenului în respirometru. *Probl. Agrofit. Teor. Aplic*., X, (4), 327-332 (1988).
14. D. B. HIBBERT and J. J. GOODING,**.** *Data analysis for chemistry. An introductory guide for students and laboratory scientists.,* Oxford University Press, Oxford, New York, 62-97 (2006).