Available online at www.sciencedirect.com
SCIENCE DIRECT
Biochimica et Biophysica Acta 1734 (2005) 215 – 219
BIOCHIMICA ET BIOPHYSICA ACTA
BBA
http://www.elsevier.com/locate/bba

Rapid report

Presence and potential signaling function of *N*-acylethanolamines and their phospholipid precursors in the yeast *Saccharomyces cerevisiae*

Olaf Merkel[a,1], Patricia C. Schmid[b], Fritz Paltauf[c], Harald H.O. Schmid[b,*]

[a]*Institute for Molecular Biosciences, University of Graz, A-8010 Graz, Austria*
[b]*The Hormel Institute, University of Minnesota, Austin, MN 55912, USA*
[c]*Institute of Biochemistry, Technical University of Graz, A-8010 Graz, Austria*

Received 1 March 2005; received in revised form 17 March 2005; accepted 21 March 2005
Available online 7 April 2005

Abstract

N-Acylethanolamines (NAEs) and *N*-acylphosphatidylethanolamines (NAPEs) are trace constituents of vertebrate cells and tissues and much is known about their metabolism and possible function in animals. Here we report for the first time the identification and quantification of NAEs and NAPEs in several strains of the yeast *Saccharomyces cerevisiae*. Gas chromatography-mass spectrometry of appropriate derivatives revealed 16:0, 16:1, 18:0 and 18:1 *N*-acyl groups in both NAE and NAPE whose levels, in wild-type cells, were 50 to 90 and 85 to 750 pmol/μmol lipid P, respectively (depending on the phase of growth). NAPE levels were reduced by 45 to 60% in a strain lacking three type B phospholipases, suggesting their involvement in NAPE synthesis by their known transacylation activity. A yeast strain lacking the YPL103c gene, which codes for a protein with 50.3% homology to human NAPE-specific phospholipase D, exhibited a 60% reduction in NAE, compared to wild-type controls. The exposure of various yeast strains to peroxidative stress, by incubation in media containing 0.6 mM H_2O_2, resulted in substantial increases in NAE. Because yeast cells lack polyunsaturated fatty acids, they offer a useful system for the study of NAE generation and its potential signaling and cytoprotective effects in the absence of polyunsaturated ("endocannabinoid") congeners.

Keywords: *N*-Acylethanolamine; *N*-Acylphosphatidylethanolamine; Yeast (*Saccharomyces cerevisiae*); Mutant strains; Oxidative stress

1. Introduction

Long-chain *N*-acylethanolamines (NAEs) and their precursors, *N*-acylphosphatidylethanolamines (NAPEs), are ubiquitous constituents of both vertebrate and invertebrate animals, higher plants and certain microorganisms such as *Butyrivibrio* sp. and slime molds (reviewed in [1–4]). NAEs often accumulate in large amounts under pathological conditions, including myocardial and cerebral ischemia [5–7], brain trauma [8] and cytotoxicity [9,10]. In contrast to the massive NAE accumulation under conditions of membrane degradation [4–8], they have also been shown to increase reversibly under nutritional or other stress [11].

The discovery that *N*-arachidonoylethanolamine (20:4 NAE, "anandamide") occurs in pig brain and binds to the cannabinoid receptor [12] has stimulated a great deal of interest in NAE metabolism in terms of endocannabinoid-mediated cell signaling (reviewed in [2,13,14]). However, in animal cells, anandamide usually represents less than 5% of total NAEs, most of which are saturated and monounsaturated (16:0, 18:0 and 18:1 NAE), and the enzymes involved in NAE biosynthesis and degradation do not appear to exhibit any substrate selectivity [2].

In both animals and plants, NAEs are produced from the corresponding NAPEs by a phosphodiesterase of the phospholipase D type [15,16]. However, while animal NAPEs are generated by transacylation of phospholipid acyl groups from the *sn*-1 position to the amino groups of phosphatidylethanolamine (PE), plant NAPEs are generated by PE *N*-acylation with free fatty acids [3]. Because almost all polyunsaturated fatty acids of animal cells are

* Corresponding author. Tel.: +1 507 437 9610; fax: +1 507 437 9606.
E-mail address: hoschmid@hi.umn.edu (H.H.O. Schmid).
[1] Present address: Laboratory for Immunological and Molecular Cancer Research, Paracelsus Medical University, A-5020 Salzburg, Austria.

1388-1981/$ - see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.bbalip.2005.03.004

esterified at the *sn*-2 position, the "transacylation–phosphodiesterase" pathway yields mostly saturated and monounsaturated amide-linked fatty acids in both NAPE and NAE [2].

Although certain biological effects have been ascribed to individual NAEs, such as *N*-stearoyl or *N*-oleoylethanolamine [17,18], all NAEs are believed to be up- or down-regulated together and most likely exert their reported anti-inflammatory, analgesic and neuroprotective effects [1,4] as a group. Attempts to determine the possible functional significance of NAEs in mammals are also complicated by the simultaneous generation of anandamide, a known cannabinoid receptor agonist.

This problem can be overcome by studying NAE metabolism and function in eukaryotic cells devoid of arachidonic acid, such as yeast. As an experimental system, yeast has a number of additional advantages such as short generation time, ease of genetic manipulation and a well established research history. Furthermore, yeast strain collections exist with deletions of all non-lethal genes, making tests for the physiological effects of the absence of their respective protein products comparatively easy.

Here we report for the first time the presence of NAE and NAPE in different strains of the yeast *Saccharomyces cerevisiae* and the effect of certain stress conditions on NAE and NAPE levels. We discuss a possible relationship between NAE levels and cell survival.

2. Materials and methods

2.1. Materials

N-Heptadecanoyl PE was prepared by reacting dioleoyl PE with heptadecanoic acid anhydride as described earlier [6]. Deuterated NAEs were prepared from 1,1,2,2,-[$^2H_4$]ethanolamine and the corresponding acyl chlorides as described [19].

2.2. Yeast strains and growth conditions

The *S. cerevisiae* strains used were W303a [20], MF17, a triple deletion of three phospholipases B (*plb1plb2plb3*) [21], in the same strain background. All other strains (Δpld1 and ΔYPL103c) were from the Euroscarf strain collection, whose strain background is BY4742 [22].

Yeast cells were grown for 48 h in a synthetic minimal medium, SMM (yeast nitrogen base w/o AA (Difco) 6.7 g/l, glucose 10 g/l, amino acid concentrate 50×, 20 ml/l). Amino acid concentrate 50× has the following composition: adenine 1 g/l, arginine 1 g/l, histidine 1g/l, leucine 3 g/l, lysine 11.5 g/l, methionine 1 g/l, threonine 15 g/l, tryptophane 1 g/l, uracil 2 g/l. YPD medium (yeast extract 10 g/l, peptone 20 g/l, and glucose 30 g/l), which is generally used for yeast cultivation, must not be used in these experiments since peptone consisting of meat hydrolysate contains polyunsaturated fatty acids, as well as the corresponding NAEs, which can be taken up by yeast cells (see below).

The cells were used as inoculum and growth continued until the optical densities (at 600 nm) indicated in the respective figures were reached. For challenge with H_2O_2 cells were grown to an optical density of 0.4, which is at the start of the exponential phase, and then challenged for 1 h with 0.6 mM H_2O_2. It is important to challenge yeast cells at the early growth phase since they become less sensitive at later stages of growth (K.-U. Fröhlich, personal communication).

2.3. Lipid extraction and analysis

Yeast cells were harvested by centrifugation (10 min, 4 °C, 6000 × *g*), washed twice with cold distilled H_2O and then disrupted with glass beads (0.3 mm diameter) in a Braun-Melsungen homogenizer for 3 min under CO_2 cooling. Lipids were extracted according to Folch et al. [23] and phospholipid content was determined by the method of Broekhuyse [24]. NAE and NAPE analysis was carried out as described previously. Briefly, to the lipid extracts were added 20 ng each of 16:0, 17:0, 18:0 and 18:1 [$^2H_4$]NAE and 19 pmol of C17 NAPE. NAEs and NAPEs were isolated with silica gel solid-phase extraction cartridges using mixtures of chloroform/methanol, 49:1 and 4:1 (v/v), respectively. NAPE samples were digested with phospholipase D from *Streptomyces chromofuscus* after the addition of d4 NAE standards. The resulting NAEs were separated by solid-phase chromatography. NAEs were converted to *tert*-butyldimethylsilyl derivatives and analyzed by GC-MS with a Hewlett-Packard 5890 gas chromatograph equipped with a 5972 mass selective detector and a 7673 autosampler [25]. The HP5MS column, 30 m × 0.25 mm i.d. (Hewlett-Packard, Palo Alto, CA), was programmed from 230 to 280 °C at 2.5 °C/min. The M-57 ions were monitored in selected ion monitoring mode. NAEs were quantified by comparison to the deuterated internal standards. Each sample was injected twice and the data were averaged. Also, mass spectra of NAEs prepared in the absence of internal standards were compared to authentic NAEs [1] for structural identification.

3. Results and discussion

3.1. NAE and NAPE in wild-type cells

We have unequivocally identified and quantified both NAEs and NAPEs in yeast (wild-type; BY4742) at levels ranging from about 50 to 90 and 85 to 750 pmol/μmol lipid phosphorus, respectively. Whereas NAE levels remained relatively constant during different stages of growth, NAPE levels decreased substantially between the early exponential and stationary phases (see Table 1 and Fig. 1).

Yeast cells contain primarily saturated and monounsaturated fatty acids of 16 and 18 carbon atoms [26] and the