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# An assessment of the ability of yeast cells to incorporate photolabile fatty acids into their membrane phospholipids in vivo

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**The photolabile fatty acids 12-azidooleic, 12-(4-azido-2-nitrophenoxy)oleic, 12-azidolauric and 12-(4-azido-2-nitrophenoxy)lauric are readily taken up in vivo by an unsaturated fatty acid auxotroph of *Saccharomyces cerevisiae*. A low level of the two lauric acid derivatives and none of the two oleic acid derivatives are incorporated into membrane phospholipids. Under certain conditions of growth in the presence of 12-(4-azido-2-nitrophenoxy)oleic acid, the nitrophenylazide group is metabolized to a product that lacks the photolabile azido group.**

Phospholipids carrying a photosensitive group which generates a highly reactive chemical intermediate upon irradiation, have been used extensively in recent years to study lipid protein interactions in artificial lipid vesicles (see Ref. 1). However, this type of study has a serious limitation in that phospholipid vesicles can only ever approximate the interactions that occur in the natural biological membrane. The incorporation of photolabile fatty acids into biological membranes would therefore considerably enhance the usefulness of the technique of photoaffinity labelling as it allows the study of the protein and lipid interactions within the membrane.

In the present study, the ability of a fatty acid desaturase mutant of *Saccharomyces cerevisiae* (strain KJ69, closely related to strain KD115 [2,3]) to incorporate two photolabile derivatives of oleic acid (12-azidooleic and 12-NAP-oleic acids), and two photolabile derivatives of lauric acid (12-azidolauric and 12-NAP-lauric acids) into its membrane phospholipids in vivo has been assessed. The incorporation of 12-NAP-oleic acid by the unsaturated fatty acid auxotroph was initially investigated because oleic acid is the major unsaturated fatty acid in yeast, and the nitrophenylazide photolabile group is considered to be of much greater potential use as a photoaffinity probe than the azide group alone. The nitrophenylazide group is readily activated by exposure to 350 nm wavelength ultraviolet light, and yeast cells have no appreciable absorption at this wavelength (data not shown).

When grown in an excess amount of oleic acid (1000 μg/ml, Table I, culture 1) the unsaturated fatty acid auxotroph grew to a final cell density of 4.6 mg cell dry weight/ml of media, and had an unsaturated fatty acid content of approx. 84% of total fatty acids when harvested. In contrast, very little growth was observed in cultures that were provided with only 12-NAP-oleic acid (1000 and 500 μg/ml) as a source of unsaturated fatty acid (Table I, cultures 2 and 3, respectively). Such cultures had significantly reduced cellular unsaturated acid contents (down to approx. 30% of

Abbreviation: NAP, (4-azido-2-nitrophenoxy)-.

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TABLE I

GROWTH AND FATTY ACID ANALYSIS OF CELLS GROWN IN 12-NAP-OLEIC ACID

Strain KJ69 was grown in the presence of nine different fatty acid supplements in media containing 1% (w/v) glucose, 1% (w/v) yeast extract and a salt mixture [10]. Free fatty acids were added into the media as a solution in ethanol to give a final ethanol concentration of approx. 2%. Cells were grown in 10 ml of media at an initial cell density of 0.2 mg cell dry weight per ml of media in 50 ml fluted flasks at 28°C by vigorously aerating on a gyrotary shaker. After 16 h growth the cell density was determined prior to extraction of the cellular phospholipids by disrupting extensively washed cells with glass beads [11] and extracting the phospholipids essentially as described by Bligh and Dyer [9]. The fatty acid composition of the extracted phospholipids was determined as previously described [3]. Strain KJ69 a *ole1* [*rho*$^+$] which carries the previously reported *ole1* mutation [2] affecting the $\Delta^9$ fatty acyl desaturase and conferring an auxotrophic requirement for unsaturated fatty acid to the mutant strain was constructed by the cytoduction procedure [12,13] from strain KD115 a *ole1* [*rho*$^+$] which was isolated by Resnick and Mortimer [2] and strain J69-1B a *ade1 his* [*rho*$^+$] from which most mutants reported from our laboratory have been isolated [14]. 12-NAP-oleic acid was synthesized as described in Table II.

| | | Culture | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
| Oleic acid supplement ($\mu$g/ml media) | | 1000 | 0 | 0 | 50 | 100 | 250 | 500 | 50 | 250 |
| 12-NAP-oleic acid supplement ($\mu$g/ml media) | | 0 | 1000 | 500 | 950 | 900 | 750 | 500 | 450 | 250 |
| Cell density (mg cell dry weight/ml media) | | 4.6 | 0.8 | 0.7 | 2.2 | 3.2 | 4.0 | 4.5 | 2.5 | 4.8 |
| Cellular unsaturated fatty acid, excluding 12-NAP-oleic acid (% of total fatty acids) | | 84.5 | 31.8 | 30.5 | 35.4 | 47.5 | 70.5 | 81.7 | 31.4 | 65.0 |
| Cellular 12-NAP-oleic acid [a] (% of total fatty acids) | (a) | 0.0 | 20.0 | 7.0 | 6.0 | 5.0 | 2.5 | 0.5 | 2.0 | 0.5 |
| | (b) | 0.0 | 0.0 | 0.0 | 0.5 | 3.5 | 4.5 | 3.5 | 1.5 | 5.5 |

[a] The cellular 12-NAP-oleic acid gas-liquid chromatography peak is split into that which (conans and (b) lacks the azido group. Both of these peaks are described in detail in Fig. 1.

total fatty acids), although a significant amount of the photolabile fatty acid (about 20% and 7% of the total cellular fatty acid for the cultures provided with 1000 and 500 $\mu$g of 12-NAP-oleic acid per ml of media, respectively) appeared to be taken up by the cells.

The addition of oleic acid from 50 to 500 $\mu$g/ml to 12-NAP-oleic acid in the growth media (from 950 to 500 $\mu$g/ml) resulted in a progressive increase in the final cell density and the cellular unsaturated fatty acid content of the cells (Table I, culture 4 to 7), but no significant increase in the incorporation of 12-NAP-oleic acid was observed. Interestingly, however, as the level of oleic acid supplement was increased relative to that of 12-NAP-oleic acid, the 12-NAP-oleic acid peak observed in the gas-liquid chromatography analysis splits into two distincts peaks (denoted peak 'a' and peak 'b' in Fig. 1). Infrared analysis of extracted phospholipids clearly shows that peak 'a' still retains the azido group (Fig. 1A), and that peak 'b' lacks the azido group (Fig. 1B).

The unexpected observation that the azido group from a photolabile fatty acid can be removed by yeast cells has two important implications. Firstly, it provides a strong indication that the 12-NAP-oleic acid was infact taken up and metabolized, and not simply adsorbed to the surface of the cells despite extensive washing. Secondly, the observation emphasises the importance in studies involving the incorporation of photolabile fatty acids into cellular membranes, to ensure that the conditions of growth and supplementation are such that any metabolism of the fatty acid is minimized, otherwise complications in the interpretation of results may occur.

Preparative layer chromatography purification of phospholipids that contain predominately the 'a' peak discussed above are shown by infrared analysis (Fig. 2a) and gas-liquid chromatography analysis (Fig. 2b) to completely lack 12-NAP-oleic acid. This clearly indicates that although a signifi-

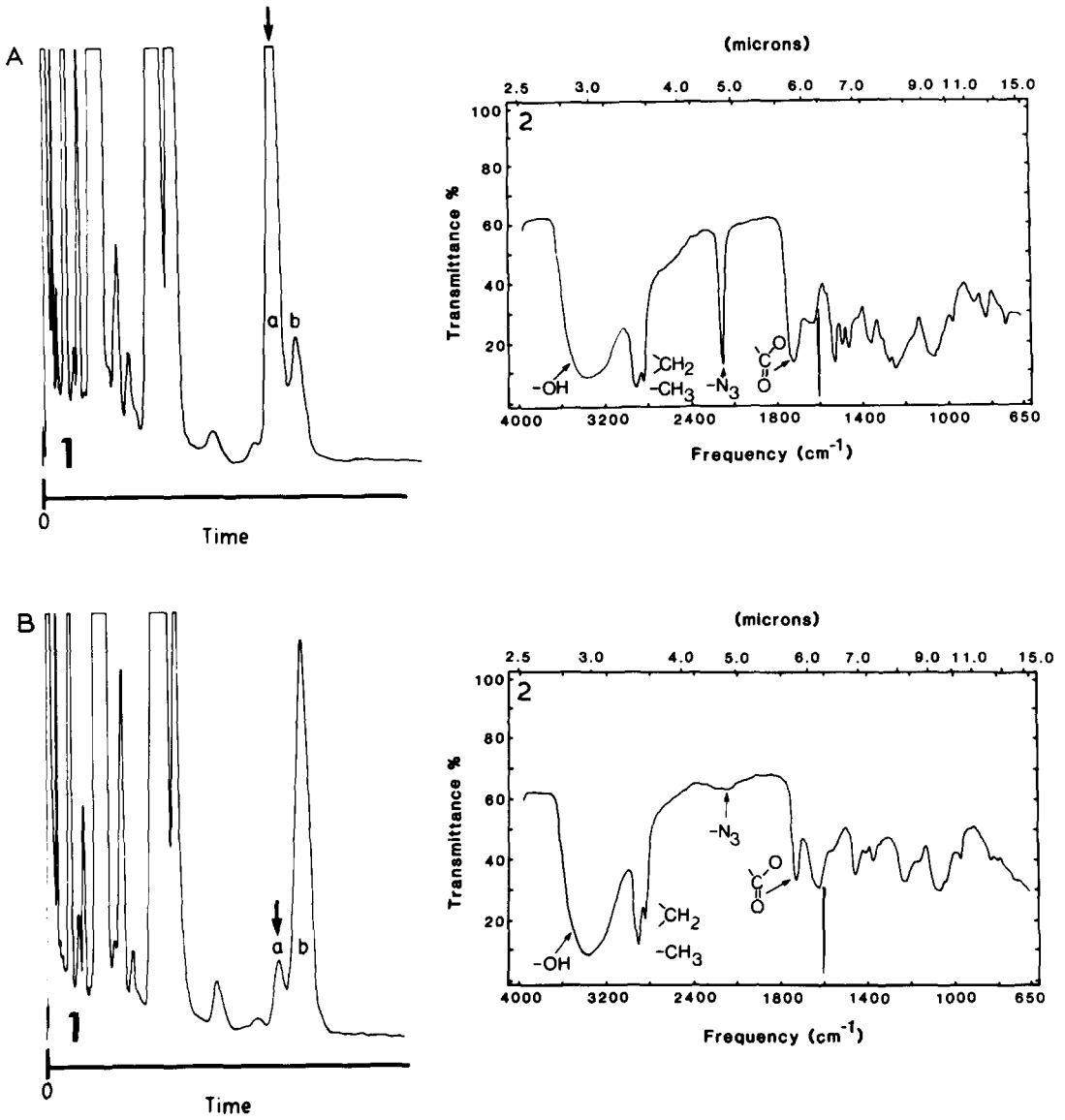
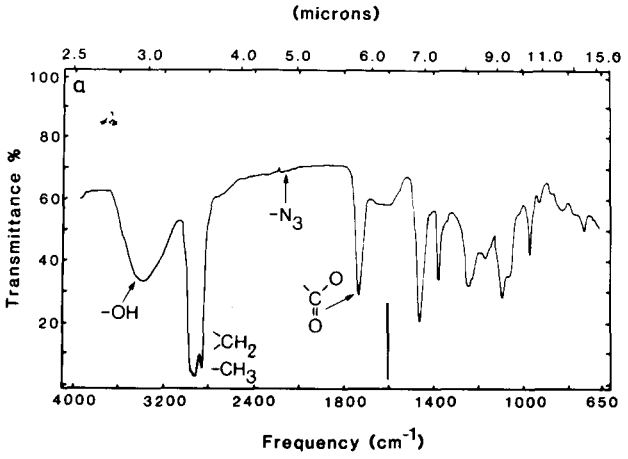



Fig. 1. Infrared spectra of the two gas-liquid chromatography peaks corresponding to 12-NAP-oleic acid found after extended growth of cells in media containing 12-NAP-oleic acid. Cells were grown in (A) 12-NAP-oleic (950 $\mu$g/ml) and oleic acids (50 $\mu$g/ml) and (B) 12-NAP-oleic (250 $\mu$g/ml) and oleic acids (250 $\mu$g/ml). After 20 h of growth the cells were harvested, the phospholipids extracted and analysed for their fatty acid composition as described previously [3] without any prior purification. Phospholipids were extracted from yeast cells by a modification of the method of Bligh and Dyer [9]. (1) Fatty acid analysis of extracted phospholipids. (2) Infrared spectra of extracted phospholipids. Two peaks, denoted peak 'a' and peak 'b' were observed in the expected position of 12-NAP-oleic acid. The position of 12-NAP-oleic acid is marked with an arrow.

cant level of 12-NAP-oleic acid is taken up by growing yeast cells, there is no incorporation of the photolabile fatty acid into the membrane phospholipids. The nitrophenylazide (NAP) group is quite bulky and thus the lack of incorporation of the oleic acid derivative into complex phospholipids may be due to the inability of one or more of the many enzymes involved in the synthesis of phospholipids and their intracellular transport to recognize this photolabile fatty acid.



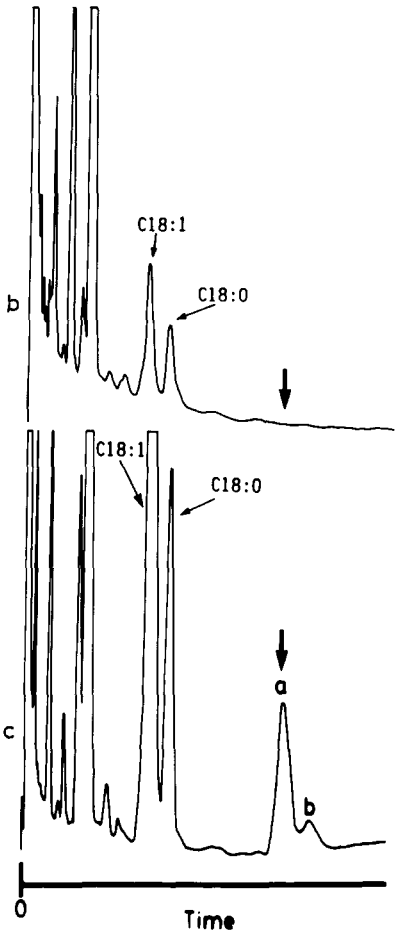




Fig. 2. Fatty acid analysis and infrared spectra of thin-layer chromatography purified phospholipids that were extracted from cells grown in 12-NAP-oleic acid. Strain KJ69 was grown in media containing 12-NAP-oleic (950 $\mu$g/ml) and a small amount of oleic (50 $\mu$g/ml) acids, and analysed for their lipid composition. (a) Infrared spectra of thin-layer chromatography purified phospholipids. (b) Fatty acid analysis of thin-layer chromatography purified phospholipids. (c) Fatty acid analysis of whole cells. The expected position of 12-NAP-oleic acid is marked with an arrow.

Since the photolabile fatty acid could not be detected in phosphatidic acid, the block in the incorporation of the fatty acid presumably occurred at or prior to the fatty acyl synthetase.

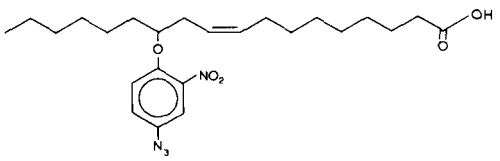
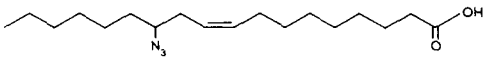
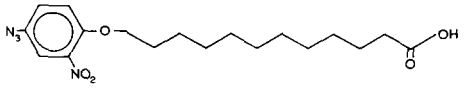
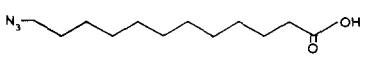
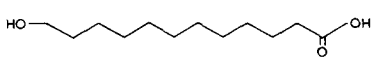
On the basis of the above considerations, three other photolabile fatty acids were investigated. The incorporation of 12-azidooleic acid into the cellular phospholipids was studied as the azido group is relatively small and so would have a minimal effect on the structure of the acyl chain. In addition, it might be possible to minimize the molecular disturbance which potentially can be created by the bulky NAP group by attaching it to a shorter 12-carbon fatty acyl chain (12-NAP-lauric acid). Lauric acid (C12 : 0) occurs in only trace amounts in the yeast cellular membranes, but 12-NAP-lauric acid has a similar molecular size to oleic acid and thus may be more readily recognized by the enzymes involved in phospholipid biosynthesis. Furthermore it may be able to closely pack into a biological membrane and at the same time maintain the essential fluidity of the membrane.

A significant level of both 12-azidooleic and 12-NAP-lauric photolabile fatty acids were found to be taken up by yeast cells (approx. 14% and 25% of total fatty acids for the 12-azidooleic and 12-NAP-lauric acids, respectively (see Table II)). Preparative layer chromatography purification of phospholipids extracted from cells grown in the presence of these photolabile fatty acids indicates that 12-azidooleic acid was not incorporated into the phospholipids (see Table II). However, a low level of 12-NAP-lauric acid (approx. 1–2% of total

TABLE II

THE UPTAKE OF PHOTOLABILE FATTY ACIDS BY YEAST CELLS

Strain KJ69 was grown in the presence of the following fatty acids at a concentration of 1000 μg/ml of media as described in the legend to Table I. After 20 h growth the cells were disrupted with glass beads and the phospholipids extracted as described in Table I. Phospholipids were separated on 2 mm thick precoated chromatography plates (Merck Art. 5554, F.R.G.) using chloroform/methanol/water (65:25:4, v/v). Further analysis of the separated phospholipids was carried out by scraping the relevant bands from the plate and extracting the phospholipids with chloroform/methanol/water (65:25:4, v/v). The level of each of the photolabile fatty acids was determined by gas-liquid chromatography as previously described [3]. The photolabile fatty acids used in the present study were synthesized essentially as described by Chakrabarti and Khorana [15]. 4-Fluoro-3-nitrophenyl azide, used in the synthesis of nitrophenylazide fatty acids was synthesized as described by Fleet et al. [16]. n.d., not determined.

| Fatty acid | Structure | | Fatty acid (% of total fatty acids) | |
|---|---|---|---|---|
| | | | Uptake by yeast cells [a] | Incorporation into membrane phospholipids [b] |
| 12-NAP-oleic |  | I | 7 | 0 |
| 12-Azidooleic |  | II | 14 | 0 |
| 12-NAP-lauric |  | III | 25 | approx. 1–2% |
| 12-Azidolauric |  | IV | n.d. | approx. 3% |
| 12-Hydroxylauric |  | V | n.d. | 10 |

[a] Fatty acid analysis of whole cells.

[b] Fatty acid analysis of preparative layer chromatography purified phospholipids.

fatty acids) was detected in purified phospholipids recovered from the preparative layer chromatography plate (Table II). In addition, fatty acid analysis of the preparative layer chromatography purified phospholipids from cells grown in the presence of 12-azidolauric acid shows that this fatty acid was also incorporated into phospholipids, albeit at a low level (approx. 3% of total fatty acids, Table II). Considering that the hydroxy group attached to the precursor fatty acid used to synthesize the above photolabile fatty acids (12-hydroxylauric acid) is much smaller than the NAP or the azido group, it is of interest that 12-hydroxylauric acid is readily incorporated into yeast phospholipids in vivo, to a level of approx. 10% of total fatty acids (Table II).

The above results are of significance as they emphasise the importance in all studies involving the incorporation of photolabile fatty acids into cell membranes to demonstrate that the fatty acid is indeed incorporated into phospholipids. There have been a number of reports in the literature describing the uptake of photolabile fatty acids by micro-organisms [4–7] as well as mammalian cells [8]. None of these reports provide sufficient evidence to demonstrate that the fatty acid is indeed

incorporated into phospholipids. Such information is critical for the application of an in vivo system to photoaffinity labeling studies.

In the only previous attempt to incorporate photolabile fatty acids into yeast membranes [4], the yeast unsaturated fatty acid auxotroph strain KD115, was shown to readily take up 12-azidooleic acid from the media to a cellular level of 25% of total fatty acids, which is consistent with our results. Unfortunately, no attempt was made to purify the phospholipids prior to the infrared and gas liquid chromatography analysis and so it is not known whether the photolabile fatty acid was incorporated into the membrane phospholipids.

While the extent of the incorporation of photolabile fatty acids into yeast phospholipids achieved in the present study is not enough to be of direct use for in vivo photoaffinity labelling, the results are in fact very encouraging; 12-NAP-lauric acid, which is readily photoactivated at long wavelength ultraviolet light, can still be incorporated into the membrane phospholipids although in a very small amount. An attempt has therefore been made to isolate mutants that are capable of incorporating 12-NAP-lauric acid more efficiently into membrane phospholipids. A number of putative mutant strains were selected and analysed for their ability to incorporate 12-NAP-lauric acid into their membrane phospholipids. Preliminary results of this investigation were promising in that a small increase in the level of 12-NAP-lauric acid was indeed detected in the membrane phospholipids of some of these strains (data not shown), although the level was still below that considered necessary for in vivo photoaffinity labelling studies. A more extensive search is necessary for the isolation of strains capable of incorporating the required level of 12-NAP-lauric acid.

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