Nuclear Magnetic Resonance Studies of the Arabinitol Metabolism and Classification of Candida Albicans
by MARK E ANDERSON

A thesis submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Biochemistry
Montana State University

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Carbon-13 nuclear magnetic resonance spectroscopy (NMR) has been increasingly applied to problems of biochemical interest. In this thesis, natural abundance C-13 NMR was used to guide an enriched C-13 study of the arabinitol metabolism of the yeast, Candida albicans. It also proved possible to classify strains of C. albicans into groups based upon their natural abundance C-13 NMR spectra.

C. albicans is an opportunistic, pathogenic yeast. In clinical and laboratory studies, detection of arabinitol has been shown to be useful. Natural abundance spectra showed that different strains of C. albicans had different internal levels of arabinitol. The cell stores of arabinitol were shown to be influenced by changes in the salinity of the growth medium and growth temperature.

These three observations guided the design of the experiments using l-C-13- and 2-C-13-glucose and l-C-13-xylose. 5 x 10^7 yeast cells/ml were suspended in yeast extract-peptone supplemented with lOmg of labeled glucose or xylose. Production of arabinitol, glycerol and ethanol were monitored using a Bruker WM-250 operating at 62.89 MHz. It was possible to determine the routes used to produce the glycerol and arabinitol by analyzing the label scrambling patterns.

The classification work was based upon the C-13 natural abundance spectra from twenty three strains of C. albicans. Two ml of cell slurry (10^10 cells/ml) were used to acquire the spectra in less than half an hour. The difference in the stores of trehalose, arabinitol and glutamate were used as the basis for classification.

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APPROVAL

of a thesis submitted by

Mark Edward Anderson

This thesis has been read by me and has been found to be satisfactory regarding content, English usage, format, citations, bibliographic style, and consistency, and is ready for submission to the College of Graduate Studies.

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ABSTRACT

Carbon-13 nuclear magnetic resonance spectroscopy (NMR) has been increasingly applied to problems of biochemical interest. In this thesis, natural abundance C-13 NMR was used to guide an enriched C-13 study of the arabinitol metabolism of the yeast, Candida albicans. It also proved possible to classify strains of C. albicans into groups based upon their natural abundance C-13 NMR spectra.

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The classification work was based upon the C-13 natural abundance spectra from twenty three strains of C. albicans. Two ml of cell slurry (10^{10} cells/ml) were used to acquire the spectra in less than half an hour. The difference in the stores of trehalose, arabinitol and glutamate were used as the basis for classification.

INTRODUCTION

Since the introduction of Fourier-transform spectrometers in the early 1970's, nuclear magnetic resonance spectroscopy (NMR) has been applied increasingly to biological and biochemical problems. The greatest advantage that NMR offers over the traditional methods, such as radiolabeling and enzyme assays, is that it is a non-invasive technique. It is possible to monitor the active metabolism of a live specimen from inside the spectrometer. A second advantage of NMR relates to the type of information obtained from the experiments. The spectra obtained can lead to the unambiguous identification of a compound and its concentration inside of the organism.

The non-invasive aspect of NMR studies of organisms is the advantage that is most often cited in justifying a series of experiments using a spectrometer. In studies in which cells are disrupted, it is difficult, and can be impossible, to determine if a particular observation is in fact what has occurred in the organism, or if what is observed is an artifact of the extraction. Unstable chemical intermediates can be destroyed by the often harsh conditions used to stop further metabolic activity. Because the organisms are not extracted during the NMR

experiment, unstable intermediates are not exposed to a possibly destablizing environment.

The NMR technique relies upon the existence of atomic nuclei that resonate at a given radiofrequency in a magnetic field. The resonant frequency of a given nucleus is determined by a combination of the applied magnetic field and the chemical environment of the nucleus. The applied field determines a frequency range and the chemical environment determines the exact frequency in that range. There are over 250 different nuclei available for analysis. The main nuclei accessible to the spectroscopist wanting to study metabolism are H-1, C-13, N-15 and P-31, but additional work has been done with such diverse nuclei as Na-23, and Cl-35 (1,2). Another factor that affects the use of NMR spectroscopy is the natural isotopic abundance of the nucleus of interest. Often, if the natural abundance is low, then the experiment is designed around the use of a labeled compound. If the natural abundance is high, then labeling is not necessary or can be difficult to do. All solution NMR techniques, regardless of the nucleus used, can detect only the smaller molecular weight molecules that are not immobilized due to some interaction. This can be an advantage or a limitation, depending upon the type of information desired by the investigators.

Proton NMR offers the highest degree of sensitivity, but it suffers from the ubiquitous presence of water in biological samples. The water resonance is broad enough and intense enough to obscure much that is chemically interesting. There are ways of getting around this, and recent work has shown that this may not always be a problem. Arus et al have looked at intact tissues using a selective water suppression pulse sequence (3,4). This method used a "jump and return" pulse sequence (5) and had the added advantage of allowing the detection of exchangeable protons. Serum, plasma, and urine analyses have been studied by proton NMR (6,7). Proton NMR has been used to follow the labeling of a carbon compound with C-13. The newer NMR techniques can be used for the acquisition of only those signals that arise from those protons attached to a labeled carbon (8). This can give a theoretical advantage in the signal-to-noise ratio (S/N) of 22:1, as well as the elimination of the water peak. A similar technique has been used to follow the incorporation of labeled acetate into its various products (9). The selectivity of the method allowed the identification of the products in the proton spectrum.

Nitrogen-15 NMR is accomplished predominantly through use of labeled compounds. N-15 has a natural isotopic abundance of 0.37%, and a sensitivity 1/1,000 that of proton. As expected, N-15 is used primarily to follow the

nitrogen metabolism of the organism. Kanamori et al investigated the effect that a nitrogen source had on the biosynthesis of glutamine and alanine in Neurospora crassa (10). N-15 NMR can be applied to other problems. Legerton et al used it to investigate the cytoplasmic and vascular pH in Neurospora crassa (11). It has also been used to make a direct measurement of the amount of crosslinking in the peptidoglycan of bacteria (12).

Phosphorus-31 NMR has the advantage of being 1/15th the sensitivity of H-1 and having 100% natural abundance. Although the 100% natural abundance precludes the use of normal isotopic labeling methods, some modern NMR techniques have been used to circumvent part of this limitation. It is possible to observe the biochemical activity associated with a particular phosphorous compound by saturating that resonance so that it cannot initially be detected, and then measuring the increase in the intensity of that resonance (13,14). The recovery of the intensity is a combination of the influx of outside nuclei and the relaxation of the original population. The difference of the two is an indication of the transfer of the phosphate groups from one population to the next population. Oxygen-17 has been used as a method of labeling a P-31 nucleus. This caused a shift in the phosphorus resonance that was used to differentiate between two possible phosphate substitution reactions (15).

P-31 NMR has been used to understand the roles of phosphorus-containing compounds in the energetics of an organism. den Hollander et al studied the effects of oxygen on glycolysis (16). Phosphorus NMR has been used to study the pH changes associated with several biologically important compounds (17), various changes occurring in blood cells (18) and the effect of light on photosynthetic bacteria (19).

Carbon-13 NMR has an advantage over the other methods of doing NMR studies because both labeled and unlabeled studies can be accomplished rather easily. Carbon-13 has a natural abundance of 1.1% and a sensitivity only 1/60 that of proton NMR. The low natural abundance means that a compound enriched to the 90%+ level in the desired position has almost two orders of magnitude greater intensity than background signals, concentrations being equal. Such a label can be followed as it passes from one intermediate to another during the metabolism of an organism. As with any labeling study, only those compounds that have both a sufficient concentration and are labeled will be detected. If the intermediate of interest does not accumulate to a level greater than the noise level, it will be missed by the experiment. If the starting material is not labeled in the proper site, then the intermediate(s) will not be labeled. Proper experimental design should avoid these obvious pitfalls, but then the investigator may miss the

unexpected. A natural abundance study does not suffer from the limitation of a misplaced label. By its very nature, all of the resonances of a compound are present. This is offset by the lack of sensitivity of the technique. The use of the newer, more sensitive spectrometers has partially overcome the sensitivity problem, but it still limits the utility of this technique when applied to biological investigations. To get around this disadvantage, it is possible to use a uniformly enriched substrate (20). The degree of labeling used is dependent upon the type of experiment. Some of the two-dimensional NMR techniques would benefit from the use of a 90%+ enrichment, but a standard one-dimensional spectrum may be overwhelmed by the interactions between neighboring C-13 nuclei. A useful level of uniform enrichment is approximately 20%. This minimizes the carbon-carbon interactions to 4% of the total number of nuclei.

C-13 NMR of biological systems has usually been accomplished through the use of labeled compounds. The analyses done using carbon-13 labeling are then analogous to those done with carbon-14. The additional advantage that NMR gives is the knowledge of the chemical structure of the compound. One of the first studies using C-13 NMR was done by Eakin et al (25). They used 1-C-13-glucose to study the anaerobic metabolism of Candida utilis. R. G. Shulman has done numerous studies using enriched

compounds to study metabolism. He and his co-workers have studied label scrambling in yeast glycolysis and trehalose production using 1-C-13-glucose and 6-C-13-glucose (21) and trehalose mobilization and utilization in yeast spores (22,23). Sacrificing the non-invasive aspect of NMR metabolic studies, Paalme, Olivson, and Vilu used the structural identification power of the technique to analyze the products of the photoassimilation and heterotrophic growth of Chlorobium thiosulfatophilum fed C-13 labeled carbon dioxide and acetate (24,25). Ogino et al studied the biosynthesis of aromatic compounds in Escherichia coli using 1-C-13-glucose (26). Since the normal concentrations of the aromatic amino acids in E. coli are below the detection limit for even a labeled C-13 study, they used mutant strains to enhance the production of these products in the pathways.

The alternative to enrichment, although not as well explored, is natural abundance spectroscopy. Natural abundance C-13 studies have become possible given the advances that high field NMR has made since the mid-1970's. The natural abundance spectra show all of the resonances due to small molecules given sufficient concentration in solution. This technique offers different information than that which is available from labeled C-13 NMR experiments. Although relatively few studies have been done using natural abundance, they have spanned a wide

range of biological systems. One of the first examples of natural abundance NMR of biological systems involved the production of adenosine-5'-triphosphate from adenosine in baker's yeast (27). In this study, Kainosho and co-workers used the presence of all of the spectroscopic peaks of the compounds present to properly identify trehalose. Trehalose had been previously observed in a study using enriched material, but was mistakenly identified as glucose-6-phosphate. R. Deslauriers et al studied the differentiation of amoeba by C-13 NMR (28). They noted the appearance of α,α-trehalose in the cells, which marked only the second time that disaccharide has been found in protozoa. One of the more intriguing studies involved the natural abundance analysis of attached grape berries (29). B. G. Coombe and G. P. Jones analyzed changes in a grape berry as it ripened over the course of three months, following the increasing concentrations of glucose and fructose and the decreasing concentrations of tartrate and malate. Lactic acid content of a frog muscle was quantified by use of natural abundance spectroscopy (30). This study showed good agreement between the NMR derived concentrations and those found by traditional, destructive methods. Additional systems studied by natural abundance spectroscopy include intact nerve tissues (31), intact muscle (32,33,34), excised rat livers (35,36) and intact rat livers (37,38,39), fruit pulps (40), live cestodes

(41), germinating soybean seeds (42), and various non-yeast fungi (43,44,45).

Definition of the Problem

The arabinitol metabolism of C. albicans was studied using natural abundance and isotopically enriched C-13 NMR spectroscopy. Other work was done to further the use of natural abundance C-13 NMR as a yeast classification technique.

Arabinitol Studies

Natural abundance spectra of C. albicans revealed an abundance of what was later identified as arabinitol. In clinical and laboratory studies the detection of arabinitol has been shown to be a possible indication of disseminated candidiasis (46,47,48,49,50,51,52). Not all cases of disseminated candidiasis, however, show positive results for the presence of arabinitol using the standard methods (56). It has been subsequently proposed that the stereoisomeric configuration of arabinitol can be used to detect disseminated candidiasis (58). Given the high internal levels of arabinitol in the yeast, I became intrigued with arabinitol production by C. albicans and attempted to elucidate some of the conditions that induced its production by the yeast.

The arabinitol metabolism of C. albicans was influenced by growth temperature, saline concentration, age of the culture and the strain of C. albicans used. Using these parameters to aid in the design of the experiments, I studied the production of arabinitol by C. albicans using labeled C-13 NMR. The yeast were fed one and two labeled glucose and one labeled xylose. The utilization of glucose and the accumulation of excretion products were followed. Samples of the excretion products and cell extracts were also analyzed. The results of these accumulations provided insight into the metabolic pathways used by C. albicans to produce arabinitol.

Natural Abundance Studies

The origin of this work is found in an attempt to apply natural abundance C-13 NMR spectroscopy to the problem of elucidating the chemical basis for the changes occurring in germinating cells of Candida albicans (65). C. albicans is an opportunistic pathogenic yeast that is responsible for a majority of nosocomial infections. Two morphological forms are associated with infection by the fungus. The first is the unicellular yeast form. The second is an aseptate mycelial form. The transition from the yeast form to the mycelial form is referred to as germination. Much work has gone into assessing the function of germination in infectivity.

From natural abundance C-13 NMR experiments done with the germinating yeast, my co-worker and I decided to test the applicability of the method to classification of the C. albicans. Since it is possible to serotype strains of Salmonella typhimurium, it has been assumed to be possible to do this with C. albicans. Hasenclever and Mitchell laid the groundwork for the antigenic studies of C. albicans (53). The importance of identifying the individual strains of C. albicans would be in the area of epidemiology. This information could be used to track the spread of an infection through a population, possibly identify the source of an epidemic, and be used to track the spread of strains resistant to the drugs used to treat infections. The spectra reported in the literature indicated that the natural abundance spectra were sensitive enough to distinguish between widely different organisms, e.g.- cestodes (41) and grapes (29). Our own experiences also indicated this. We gathered spectra on over twenty different strains of C. albicans, and from these spectra a pattern emerged. By assigning the resonances of the spectra to various compounds, it was possible to derive a statistical basis for the visual classification scheme that we had developed earlier that had divided strains of C. albicans into similar groups.

EXPERIMENTAL

Organisms

The yeast used in the NMR experiments were obtained from several sources. Candida albicans strains 16-149 were obtained from the Clinical Microbiology Laboratories, University Hospital, University of Washington, Seattle, WA. C. albicans strains 158-164 were a gift from Dr. T. G. Mitchell (Duke University, NC). All strains were clinical isolates passed in culture no more than twice before being received. C. guillermondi, C. krusei, C. pseudotropicalis, C. tropicalis, and Torulopsis glabrata were from the Montana State University mycological culture collection.

Preparation and Maintenance of Organisms

Natural Abundance Experiments

For the yeast, storage was on potato flakes agar (PFA) slants at 4-6°C during the initial portion of the study and later on Sabouraud's dextrose agar slants at room temperature. Biochemical confirmation of all species was performed using API strips from Analytab Products, Ayerst Labs (Plainview, NY). Cells for NMR use were grown on

synthetic amino acid medium for fungi (SAAMF) (Table 1). The medium was prepared in accordance with the instruction provided by Gibco (Grand Island, NY) with the medium. This included the addition of 10g/l of glucose followed by filter sterilization.

For a natural abundance NMR experiment, the cells were inoculated into 2 ml of SAAMF in a 5 ml tube and incubated for 24 hours at 37°C with aeration provided by constant rotation at 160 rpm. One hundred and fifty ml of SAAMF in a 250 ml Erlenmeyer flask was inoculated with two drops of the overnight culture and incubated at 37°C, with 160 rpm for 24 hours. Cells obtained for the 28°C temperature experiments were grown in a shaking water bath rotating at 160 rpm. Cells grown on GYEP for the salt supplement experiments, followed the same protocol as the cells grown on SAAMF. Cells were collected, washed three times in 0.15 N NaCl and 0.5 - 2 x 10^{10} cells were packed into a 10 mm NMR tube. Cells were kept on ice until use.

Enriched Experiments

The yeast were maintained by the same methods as used for the natural abundance experiments. The inoculum was grown for 24 hrs. in 2 ml of GYEP at 37°C. One drop was then transferred to 10 ml of GYEP in a 25 ml Erlenmeyer. The culture was grown for 24 hrs. on a shaker at 160 rpm at 37°C. The cells were harvested, washed in sterile distilled water three times, and then kept refrigerated

Table 1. Formulation for Synthetic Amino Acid Medium Base, Fungal

Ingredients[a]	Amount (g/l)
L-arginine	1.05
L-lysine	0.58
L-histidine	0.31
L-tyrosine	0.36
L-tryptophan	0.36
L-phenylalanine	0.32
L-cysteine	0.24
L-methionine	0.15
L-threonine	0.48
L-leucine	0.52
L-isoleucine	0.52
L-valine	0.46
fumaric acid	1.50
Na-pyruvate	1.00
NH_4-acetate	0.50
$K_2HPO_4 \cdot 3H_2O$	0.50
L-glutamine	2.52
L-aspartamine	1.00
L-proline	1.00
glycine	0.50
MOPS[b]	16.45
TRIS[c]	10.45

Table 1 (continued)

Ingredients[a]	mg/l
biotin	0.50
folic acid	5.00
choline chloride	25.00
nicotinamide	5.00
di-Ca pentothenate	25.00
pyidoxal HCl	5.00
thiamine HCl	5.00
riboflavin	5.00
i-inositol	25.00
$MgCl_2$ anhydrous	95.35
$FeCl_3 \cdot 6H_2O$	2.70
$ZnSO_4 \cdot 7H_2O$	0.80
$MnSO_4 \cdot 4H2O$	0.36
$CaCl_2$ anhydrous	5.57
phenol red	2.00

[a] All chemicals came premixed in one packet from GIBCO (Grand Island, NY)

[b] MOPS = 2-(N-morpholino)propane sulfonic acid

[c] TRIS = 2-amino-2-(hydroxymethyl)-1,3-propanediol

until use. It was found that the cells could be kept at 4°C for up to one week without affecting the results of the experiment. For the actual experiment, the cells were counted, suspended in sterile distilled water, and added to the NMR tube just before use. Final concentration in the NMR tube with the addition of the 1.5 ml of medium was 5 x 10^7 cells/ml for most experiments. The one exception used $1x10^9$ cells/ml.

The 10 mm NMR tube contained 0.50 ml of sterile 1.20 N NaCl in D_2O, 1.00 ml of sterile 1.0% yeast extract-2.0% peptone solution and 10.0 mg labeled sugar. This solution was mixed up to thirty minutes prior to the NMR run. The 10 mm NMR tube was moderately sterile in that it had been washed with a germicidal detergent and was then dried in a culture transfer hood under germicidal UV light.

NMR Parameters

All experiments were done on a Bruker WM-250 spectrometer operating in Fourier transform mode with quadrature detection. The operating frequency was 62.89 MHz. The spectrometer was interfaced to an Aspect 2000 computer. All data files were of 8k length. The natural abundance spectra were transformed using a 10 Hz line broadening factor applied by an exponential multiplication. The enriched experiments were transformed using a 5 Hz line broadening factor.

Natural abundance spectra were acquired while the temperature of the sample was maintained at 2°C ± 2° by means of the standard cooling equipment available with the Brker WM-250. The enriched spectra were acquired while the temperature was maintained at 28°C +/- 2°. Some overheating problems were found to have occurred due to the power of the broadband decoupler. This difficulty was overcome by use of a bilevel decoupling routine. The routine switched the power level from 2 watts during the acquisition of the spectrum to 0.2 watts during the delay period.

For both sets of experiments, the sweep width was from -10 to 220 ppm. The lock signal for the natural abundance experiments was provided by either a D_2O/TMS capillary, a d_6-benzene capillary or one of two external sources, a D_2O/ethanol sample or a $CDCl_3$ sample. For all of the natural abundance experiments except the saline experiments, the pulse width was 85°, the relaxation delay was 3.0 seconds and the acquisition time was 0.3 seconds. Six hundred scans were accumulated per file. For the saline experiments, the pulse width was 45° and the relaxation delay was 1.5 seconds. One thousand scans per file were accumulated. The enriched experiments used the same pulse width and delay as the saline experiments except that only 500 scans per file were accumulated. The lock signal for the enriched experiments was provided by the D_2O

in the 0.5 ml of 1.200 N NaCl solution that was added to the medium in the NMR tube.

Oxygen was provided by a pair of capillaries (Fig. 1). The design of this system was suggested by the coaxial system developed by den Hollander for use in his experiments (54). Two 2mm diameter glass tubes were used. The longer, finer capillary was coaxial and went to the bottom of the 10mm tube. Its function was to provide mixing and oxygen in such a way as to minimize field perturbations. This tube had a bubbling rate of approximately 2 ml/min. The larger diameter tube was not coaxial, but was above the sensing coils of the NMR. It provided a high rate of oxygen bubbling (20 ml/min) with larger bubbles. The two tubes were held in place by a modified Teflon vortex plug.

Due to loss of fluid during the experiments, it became necessary to bubble the oxygen through an Erlenmeyer flask half filled with heated water (40°C).

Identification

Natural Abundance Experiments

Identification of the compounds was accomplished by comparison of the resonances in the experimental spectra to spectra of known compounds.

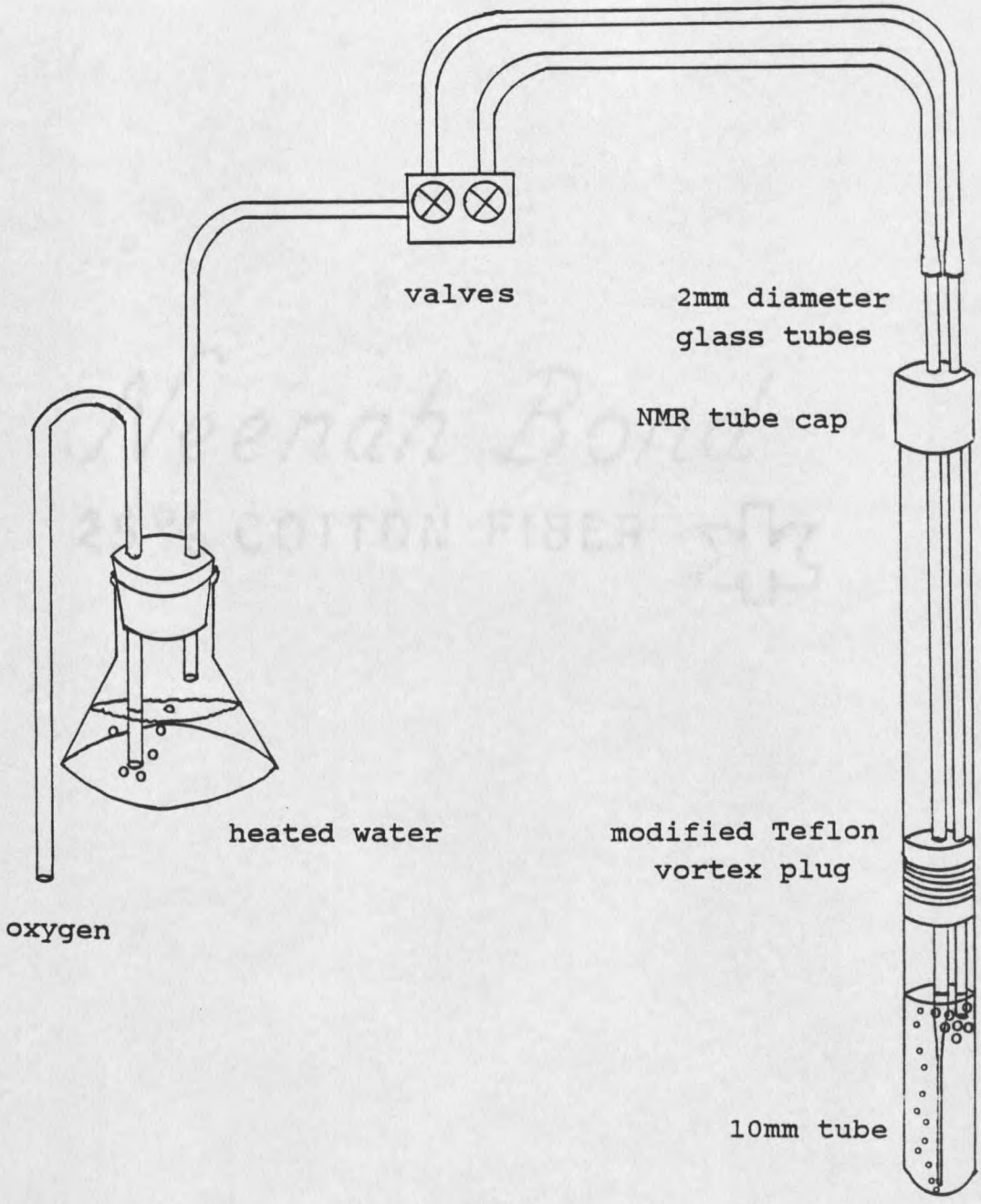

Figure 1. Apparatus used to bubble oxygen into the 10 mm NMR tube during the data acquisition.

Enriched Experiments

At the point during an enriched experiment when the glucose was nearly depleted, the NMR tube was removed from the spectrometer and put on ice. The cells were then immediately separated from the supernatant liquid by thrice repeated centrifugation and washing. The supernatant liquid and washings were combined and filtered through 0.45 μm Millipore filters. One sample was freeze-dried to reconcentrate it. Methanol was added as an internal reference and then analyzed on the NMR using the same parameters as those used during an enriched experiment except that the temperature was uncontrolled but remained near 22°C. The resulting spectra were compared to the resonances obtained from external standards.

The cells were harvested by centrifugation and the cell walls were fractured by use of the glass bead technique (55). The fractured cells and their contents were filtered on 0.45 μm Millipore filters and the filtrate was freeze-dried and then analyzed using the same protocols as the supernatant liquid samples.

RESULTS

Natural Abundance Carbon-13 Studies of Candida albicans

Figure 2 is a spectrum typical of those obtained by natural abundance C-13 NMR. The example is a spectrum of the yeast Candida albicans showing the transformed spectrum from 0-200 ppm. The line widths of the peaks ranged from 10-15 Hz.

The assignments of the resonances of this and other spectra of the yeasts were confirmed by comparison to external standards. The standards were run under conditions that as closely as possible mimicked those of the experiment. The identifications were cross-checked by comparison to reports of high concentration compounds in yeast and particularly C. albicans. Sullivan et al extracted cells of C. albicans and identified some of the contents by the standard methods of chromatography (56).

The region from 0-55 ppm is dominated by the resonances from free amino acids. The largest peaks are due to the methylene resonances of glutamic acid at 27.2, and 33.5 ppm and the α-carbon at 54.9 ppm. Often associated with these peaks are the analogous resonances of glutamine at 26.7,

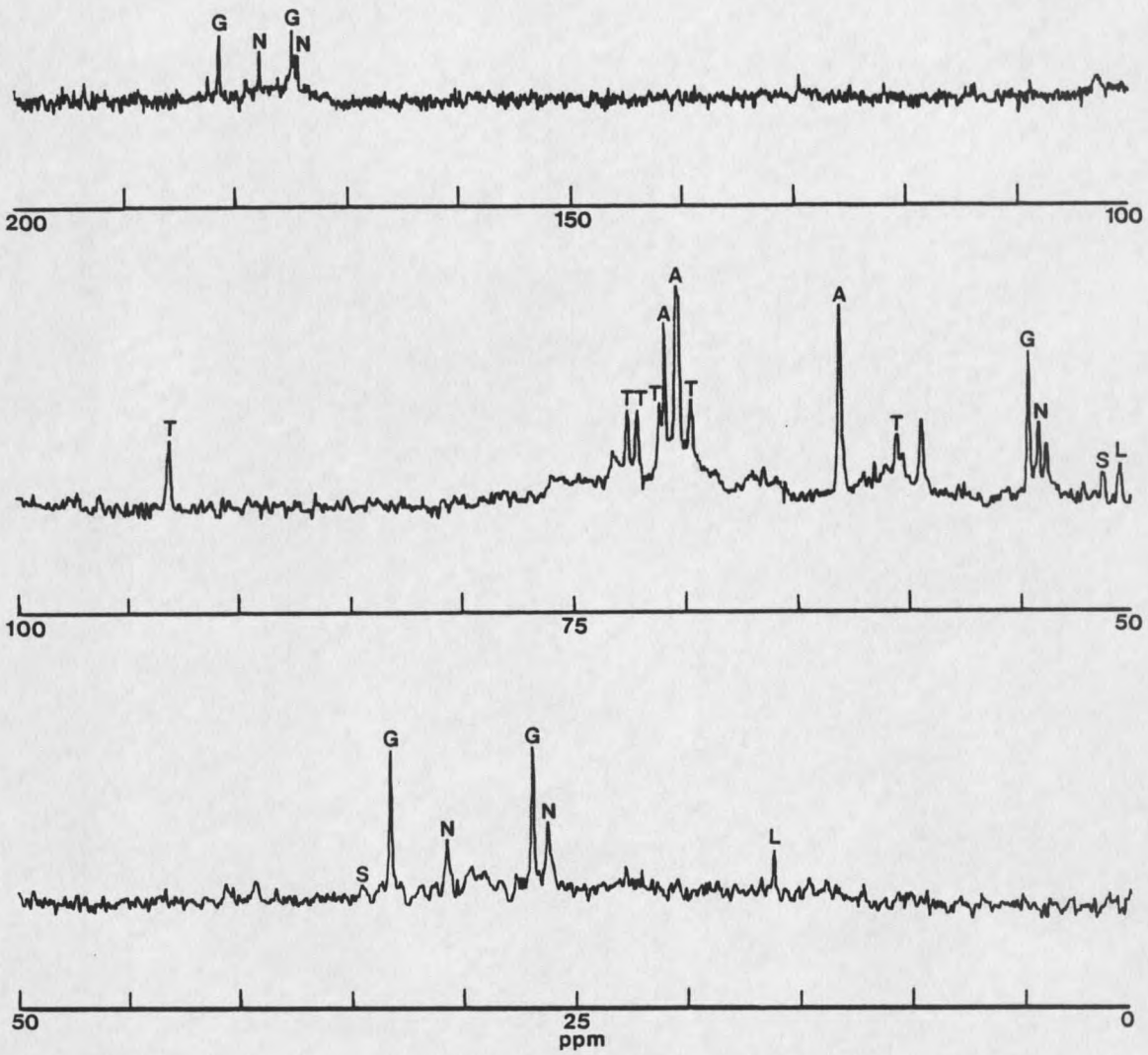

Figure 2. C-13 NMR spectra of C. albicans strain 118 grown 24 hours on SAAMF. The abbreviations are: A arabinitol, G glutamate, N glutamine, Y glycerol, L alanine, T trehalose, R aspartate, P proline and S aspartamine.

31.1, and 54.4 ppm. Alanine, another amino acid that is frequently seen, resonates at 16.4 and 50.9 ppm.

Occasionally visible in other spectra are proline at 24.1, 29.4, 46.3 and 61.3 ppm and ethanol at 17.1 and 57.8 ppm.

In the region from 55-110 ppm, the major resonances are due to the alcohol groups on carbohydrates, their hemiacetals and hemiketals, and the amino carbons of some compounds. One of the major carbon compounds found in C. albicans is arabinitol. It dominates this region of the spectrum with its resonances at 63.3, 63.4, 70.6, 70.8 and 71.3 ppm. Typically the resonances for the C-1 and C-5 carbons at 63.4 and 63.3 overlap to such an extent that they cannot be resolved into separate peaks. This is often true of the C-2 and C-3 resonances at 70.6 and 70.8 ppm. Although the intensities of the individually resolved resonances should be equivalent, overlap with neighboring resonances and the use of an exponential line broadening technique can alter the relative heights. Another polyol detected in Candida albicans was glycerol, with peaks at 62.8 ppm for the C-1 and C-3 carbons, 72.4 ppm for the C-2 carbon. Ribitol, another five carbon polyol, also has resonances at 62.7, 72.3 and 73.6 ppm. Glycerol was chosen as the compound responsible for the resonances seen in the spectra because of the relative intensities of the peaks. The glycerol 62.8 peak would have an intensity

approximately twice that of the 72.4 peak. Ribitol would have nearly equal intensities for all peaks. The other major carbohydrate component in the cells is trehalose. Trehalose is an α-1,1-diglucoside. It has resonances at 93.3, 72.8, 72.4, 71.3, 69.9 and 60.7 ppm. Not all of the resonances were visible in all spectra due to overlap with neighboring resonances.

There is also a resonance at 59.4 ppm. In some spectra, this resonance is almost four times the height of any other peak in the spectra. No reasonable assignment could be made for this resonance.

Resonances in the area of 110-140 ppm are due to the aromatic amino acids and to the unsaturated fatty acids (44). These peaks are usually not very well defined and are often below the noise level. The region out to 200 ppm is dominated by the carboxylate resonances from the amino acids and esters of the fatty acids. These occur from 165-180 ppm.

Most of the compounds of interest and most of the changes in the spectra associated with these compounds occur in the region from 0 to 100 ppm. For this reason, all of the following spectra will span only this region.

C. albicans was studied in more detail by analyzing a total of 23 strains using C-13 NMR. For the baseline data, the growth parameters were kept the same for all of the strains. The cells were grown on SAAMF for 24 hours at

37°C. SAAMF was chosen because it is a chemically-defined medium and should not have any batch to batch variations.

Under the same conditions of growth, the strains of C. albicans can be divided into three groupings, type I, type II and type III, based upon their natural abundance spectra (Fig. 3). The type I classification encompassed the largest number of strains, seventeen. Strain 118 was arbitrarily chosen as the archetypical strain. The type II grouping was the second largest with 5 organisms. Strain 122 was chosen as the typical organism of this group. The type III group was represented solely by strain 117, and was formed by default since strain 117 fit neither the type I nor the type II spectral patterns.

Type I organisms distinguish themselves by the abundance of peaks their NMR spectra reveal, and the relative equality in the intensity of those peaks (Fig. 4). As previously mentioned, glutamate, glutamine, and alanine make up the major resonances in the region from 0-55 ppm. Arabinitol and trehalose dominate the carbohydrate region.

The type II strains are also unique in that the arabinitol peaks at 63.3, 70.4 and 70.9 ppm dominate the entire spectrum (Fig. 5). The higher concentration of arabinitol is the cause of the apparent higher signal to noise of type II spectra compared to those of other types. The concentration of glycerol is very low in type II cells. Trehalose is also present, but it also has a low