

compounds which has a relatively symmetric electronic environment. The next metabolites were detected and identified in  $^{14}\text{N}$  NMR spectra of brain: trimethylamine compounds such as choline, acetylcholine, phosphatidylcholine, sphingomyelin, several amino acids (predominantly glutamine, glutamate, asparagin, aspartate and glycine) and ammonia  $\text{NH}_4^+$ . These trimethylamine compounds as well as several amino acids are difficult to resolve in spectra. This fact is due to: a) broad lines due to tissues heterogeneity, b) overlap of lines with different  $T_2^*$ . Based on their  $^{14}\text{N}$  NMR parameters ammonia may prove to be the most practical compounds for study nitrogen metabolism in brain. Note that brain ammonia is an important byproduct of the metabolism of the putative neurotransmitters glutamate and aspartate and of the neurotransmitter monoamines. It is also the product of the synthesis of glutamate from glutamine in nerve endings.

Thus a typical  $^{14}\text{N}$  NMR brain spectrum only shows resonances from a limited number of relatively concentrated ( $>10$  mMol/l) low molecular weight metabolites. Many nitrogen-containing metabolites are unmeasurable because their concentrations fall below 1—10 mMol/l limit. Moreover  $^{14}\text{N}$  NMR measures the mobile fraction of the metabolite that contributes to the high resolution signal in the spectrum. The fraction of the metabolite that is immobilised by precipitation or by tight binding to macromolecules, membranes or organelles is undetectable.

It is necessary to note that an important aspect of the technique is the pH dependence of the  $\text{NH}_4^+$  chemical shift making it possible to estimate intracellular pH non-invasively.

In conclusion our results showed that nitrogen- $^{14}$  NMR spectroscopy allows to monitor in vitro many nitrogen-containing metabolites in brain, to measure active (non-bound) metabolites and to carry out study of biochemical changes.

## $^{14}\text{N}$ AND $^{31}\text{P}$ NMR *IN VIVO* STUDIES OF BACTERIAL CELL SUSPENSIONS

*S. Tyukhtenko* (chair of chemistry NaUKMA),  
*M. Patrauchan* (A. Dumansky Institute of Colloid and Water  
 Chemistry),  
*S. Afonin* (A. Paladin Institute of Biochemistry)

Application of instrumentation-based techniques for studying of microorganisms has been increased rapidly for the last decades. The overwhelming majority of biochemical and biophysical bacterial cells

investigations use that kind of techniques involving destructive or deep-perturbing analytical methods.

We have used  $^{14}\text{N}$  and  $^{31}\text{P}$  NMR spectroscopy as nondestructive and noninvasive method for *in vivo* studying of biochemical properties of surfactant degrading bacteria.

We have obtained *in vivo*  $^{31}\text{P}$  and  $^{14}\text{N}$  NMR spectra of several cationic surfactants (CSA) degrading bacterial cultures (genus *Bacillus*) as well as of some wild-type strains cultures (genera *Pseudomonas*, *Bacillus* and *Sarcina*).

We believe to use  $^{14}\text{N}$  NMR as a novel bacteria studying spectroscopic tool, taking into account that there are nearly almost now examples of similar application.

$^{14}\text{N}$  NMR Intracellular nitrogen-containing metabolites such as ammonium (-355...-357 ppm), free amino acids (-333...-345 ppm) and phosphatidylcholines (PC) (-328...-330 ppm) were detected and identified in  $^{14}\text{N}$  NMR spectra. Metabolites identification was performed on the basis of  $^{14}\text{N}$  NMR spectra of model compounds and according to characteristic chemical shifts and linewidths. Thanks to unique nearly spherical symmetry around nitrogen-14 nucleus, this metabolites give rise to a sharp  $^{14}\text{N}$  NMR resonance lines; hence, this allows the detection and quantification of relative  $\text{NH}_4^+$  and PC level.

$^{31}\text{P}$  NMR  $^{31}\text{P}$  NMR spectroscopy was used to detect the presence of phospholipids, lipopolysaccharides (LPS) and to assess the bioenergetic status of the cells. The  $^{31}\text{P}$  NMR spectra obtained from bacterial suspensions contained resonance lines of sugar phosphates (0...+4 ppm), inorganic phosphates (-3...0 ppm), phospholipids (-1,5...0 ppm), ADP, ATP b-phosphates (-10...-11 ppm) and well-detectable wide signal of LPS for Gram-negative bacteria. The resonances were assigned to specific metabolites on the basis of chemical shifts and linewidths values.

*In vivo*  $^{14}\text{N}$  and  $^{31}\text{P}$  NMR spectroscopy of bacterial cells have demonstrated strong evidence for the fact that all the CSA degrading strains being Gram-negative bacteria contained both LPS as typical Gram-negative and PC as typical Gram-positive, showing some-how intermediate structural features, probably leading for their cells being less sensitive to xenobiotic-rich environment.