Fri-S38-22

A 31P NMR STUDY OF SIMULTANEOUS CHANGES IN WORK AND THE PHOSPHATE POTENTIAL IN THE RAT HEART

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The relationship between changes in work (and thus oxygen consumption) and the cytosolic phosphate potential (PΨ) has been directly monitored in a 31P NMR study of the Langendorff perfused rat heart. Hearts were perfused at 37°C with 11 mM glucose phosphate-free Krebs-Henseleit buffer while left ventricular pressure and heart rate were continuously recorded. Metabolic levels were measured (μmol/g. wet wt ± S.E.M.) from spectra accumulated after 45 min perfusion: P 1.56 ± 0.24, PCR 4.33 ± 0.29, ATP 4.1240.32. The PP is estimated as 38,000 M⁻¹. Adrenaline (5x10⁻⁶M) increases the pressure-time integral by 84 ± 14% and the PP decreases to 21,000 M⁻¹.

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THE DYNAMIC STRUCTURE OF CELL-WALL COMPONENTS IN B. SUBTILIS RodA AND RodB MUTANTS AS PROBED BY 15N-NMR SPECTROSCOPY

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The structural and dynamic properties of cell envelope components of rodA and rodB mutants (morphology conditional mutants) of B. subtilis with or without treatment with mitomycin C and chloramphenicol have been examined. The bacteria were grown in Spizizen's minimal medium enriched with 15NH₄Cl. Intact cells, cell walls and the products of lysozyme digestion have been tested.

The significance of the observed modifications in the spectral parameters for the cell physiology will be discussed.

Fri-S38-25

SHAPE CHANGES IN B. SUBTILIS MORPHOLOGICAL MUTANTS IN VIVO 15N-NMR STUDIES

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A novel technique, that consists of monitoring the relative intensities [15N-H] NOE 15N-NH of bacterial cell components in the spectra of 15N-enriched whole cells and cell wall preparations has been used. Two classes of rod mutants of Bacillus subtilis were studied: Rod A mutants (200 and 230) and their parent strain, and Rod B mutant (104). These experiments provide insight into the three dimensional arrangements of cell wall polymers, which aid in the formation of a comprehensive theory of the molecular basis of cell morphology, cell wall biosynthesis and turnover, and the lethal action of antibiotics. Examination of the fine structure of the peptidoglycan from both Rod A and its autolytic deficient mutant, by 15N-NMR, indicate conformational changes around linking D-Ala residues as a result of a decrease in tetrachlor acid concentration.

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NITROGEN-15 NMR IN VIVO STUDIES OF CELL WALL SHAPE AND FUNCTION OF STREPTOCOCCUS FAECALIS

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Motional properties of bacterial cell wall polymers were studied by a novel technique that consists of monitoring the relative intensities of 15N-[H] NOE enhanced 15N-NMR resonances of cell wall components. Using this technique we are currently studying the relationship between the arrangements of cell wall polymers, in S. faecalis and in its autolytic deficient mutant Lty-14, and their morphological features. The effects of inactivation of cell wall autolysis were investigated. Exposure of exponential culture phase of S. faecalis to mitomycin C resulting in elongated cells. In contrast, the protein synthesis inhibit by chloramphenicol leads to wall thickening. These phenomena have been demonstrated by 15N-NMR. 15N-NMR was used to probe the changes in the mobility of cell wall polymers that occur under conditions leading to disruption of cross-links or bridge regions of the peptidoglycan.

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Photo-CIDNP NMR of proteins probes the surface accessibility of tyrosyl, tryptophanyl and histidyl residues. It takes advantage of a specific enhancement of the NMR-signals of these residues, provided that they are located at the surface of the protein. This enhancement is caused by the photo-dynamic interaction between irradiated flavin and one of these amino acids. By monitoring the 1H-NMR difference spectrum we have shown, that the translational factor EF-Tu-GDP from E. coli exposes 1 histidyl and 2 or 3 tyrosyl residues and that the tryptophanyl residue is completely shielded. Comparison of the spectrum of EF-Tu-GDP with that of EF-Tu-GTP did not reveal major differences. In contrast the EF-Tu-GDP from B. stearothermophilus showed only one class of tyrosyl residues on its surface.