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LYSODEKTOSE - A NEW TRISACCHARIDE FROM MICROCOCCUS LYSODEIKTICUS WHICH IS TRANSFORMED INTO AN AMINOXYL FREE RADICAL WITH THE LOSS OF AN ELECTRON

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A new trisaccharide 6-0-(2-deoxy-2-/N-methyl/-hydroxylamino-β-D-glucopyranosyl)-α,α-trehalose named lysodektose has been isolated from *Micrococcus lysodeikticus*. In oxygenated solutions or in the presence of K_3 Fe $(CN)_6$ lysodektose is transformed into a long lived free radical. Spin trapping data are presented and functions are suggested for the substance.

KEY WORDS: lysodektose, *Micrococcus*, aminoxyl radical, trisaccharide.

INTRODUCTION

Upon the addition of potassium ferricyanide to methanol extracts of certain bacteria a multiplet ESR signal can be observed with $g = 2{,}005$.[1,2] The intensity of the signal (the largest is in the extracts from *Brevibacterium ammoniagenes* and *Micrococcus lysodeikticus* of the bacterial species tested) in the case of *M. lysodeikticus* is a function of oxygen supply to the growing culture. The elongated life time of the radical is characteristic of spin adducts,[3] hence this observation is of interest for microbial physiology and biotechnology. In the present paper we describe the chemical nature of the substance isolated from *M. lysodeikticus* and named lysodektose which is transformed into a long lived free radical with the loss of an electron.

MATERIALS AND METHODS

M. lysodeikticus 2665 Fleming strain was grown at 30° in shaker flasks in peptone plus yeast extracts medium until stationary phase was reached, spun down and treated with 50% methanol solution as described previously.[1] Then the extract was concentrated *in vacuo* to 0.3 volume, passed through a column of DEAE cellulose to remove anionogenic admixtures, through a Sephadex G-15 column and finally through Silasorb C-18. An ESR signal arising on addition of up to 1 mM potassium ferricyanide to the samples was monitored throughout the separation procedure at 20°C.

A Bruker WM-250 or AM-300 spectrometer was used for NMR investigation of

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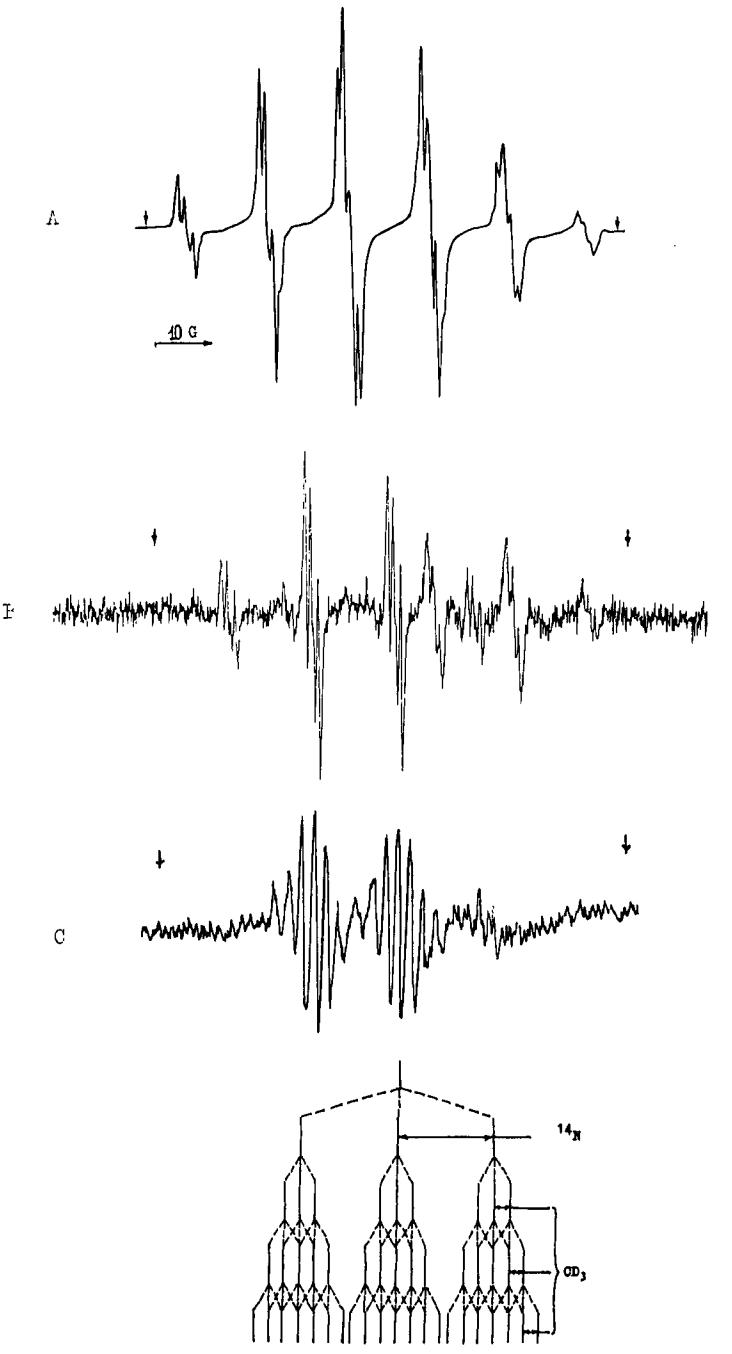

FIGURE 1 ESR spectra of extracts from *M. lysodeikticus* in presence of K_3 Fe $(CN)_6$ ~ 10^{-4} M: A - natural isotopic content, B - ^{15}N-substituted material; C - D-substituted material. Spectrometer settings: microwave power, 20 mW; modulation amplitude, 0.2 G; sweep rate, 0.3 G/s; time constant, 0.3 s. The markers position corresponds to the 3d and 4th lines of Mn^{++}/MgO.

D_2O-dissolved samples; ESR spectra were obtained on a Soviet RE-1306 spectrometer operating at 9.4 GHz with Mn^{++}/MgO as built in standard; mass spectra were recorded on a Soviet MI-1201-E where glycerol is used as a sample matrix and argon as a carrier.

When incorporation of ^{15}N was required bacterial cells were cultivated in a medium containing, per litre of tap water: $(^{15}NH_4)_2 SO_4$ - 5 g, glucose - 10 g, NaCl - 50 mg, $K_2 HPO_4$ - 100 mg, $MgSO_4 \cdot 7 H_2O$ - 50 mg, $CaCl_2 . 2 H_2O$ - 30 mg, biotin - 30 μg at pH 7.4. Deuterated medium was prepared by hydrolysis of deuterated Chlorella biomass as described.[4]

RESULTS AND DISCUSSION

The growth of *M. lysodeikticus* on ^{15}N- or deuterated media was very poor but the ESR spectra of methanol extracts of the resulting biomass revealed clearly the incorporation of both ^{15}N and D into a paramagnetic center. The characteristic splitting of the spectral lines due to the difference of spin numbers and magnetic moments of the isotopes corresponded to interaction of the unpaired electron with one N and 3 equivalent hydrogen nuclei. The small additional splitting with two non-equivalent protons ($aH_4 \sim .2{,}1$ G, $aH_5 \sim 1{,}0$ G) probably takes place at the C2 and C1 atoms of the C-ring (Figure 2). The ESR spectra of normal and isotopic substituted materials are presented on Figure 1. The following values of constants are calculated for the spectra:

$a(^{14}N) = 14{,}7$ G, $a(CH_3) = 15$ G;
$a(^{15}N) = 20{,}6$ G, $a(CH_3) = 15$ G;
$a(^{14}N) = 14{,}7$ G, $a(CD_3) = 2.3$ G

The ^{13}C-NMR and ^{1}H-NMR spectra of purified lysodektose are typical of carbohydrates and, as the largest molecular mass revealed by mass-spectroscopy is 533, a trisaccharide structure is anticipated for the substance. The detailed structure of the pyranose rings was determined by selective homonuclear double resonance following published recommendations[5-7] (Tables I and II). Thus we think that lysodektose is 6-0-(2-deoxy-2-/N-methyl/-hydroxylamino-β-D-glucopyranosyl)-α-α trehalose and is

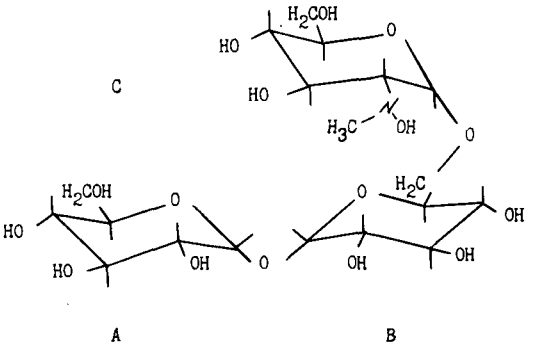

FIGURE 2 Chemical structure suggested for lysodektose from *M. lysodeikticus*.

TABLE I
^{1}H-NMR data of lysodektose

	Chemical shift ppm (J (m-n) Hz)	
Cycle A		
H-1	5.19	(d; 4.0 (1–2))
H-2	3.63	(dd; 10.0 (2–3))
H-3	3.84	(t; 10.0 (3–4))
H-4	3.76	(ddt; 9.0 (4–5))
Cycle B		
H-1	5.19	(d; 4.0 (1–2))
H-2	3.65	(dd; 10.0 (2–3))
H-3	3.86	(t; 10.0 (3–4))
H-4	3.56	(dd; 9.0 (4–5))
Cycle C		
H-1	4.85	(d; 8.5 (1–2))
H-2	2.61	(dd; 10.0 (2–3))
H-3	3.81	(dd; 9.0 (3–4))
H-4	3.44	(dd; 10.0 (4–5))
-CH_3-	2.91	

TABLE II
^{13}C-NMR data of lysodektose

Cycle A	Chemical shift ppm	Cycle B		Cycle C	
C-1	94.7	C-1	94.7	C-1	101.4
C-2	72.3	C-2	72.3	C-2	47.6
C-3	73.8	C-3	73.9	C-3	73.5
C-4	71.0	C-4	71.0	C-4	71.4
C-5	72.9	C-5	71.9	C-5	76.8
C-6	61.9	C-6	69.0	C-6	62.2
				NCH_3	34.1

transformed into a paramagnetic form by oxidation of $-\underset{CH_3}{N}-OH$ into $-\underset{CH_3}{N}-O^{\cdot}$

Among microbial metabolites there are many bearing similar chemical groups in their molecules[8–11] but we have found no information on the free radical forms of those substances.

No ESR signal is registered in a suspension of intact bacterial cells while weak signals do appear on lyophilization or treatment with methanol. In a model system containing a superoxide anion generator and detector (adrenalin) lysodektose prevented the adrenalin oxidation by $O_2^{-\cdot}$, but its real function within the mother cell is still obscure. We suggest that the substance under study is a part of the molecular mechanism of protection of the cell against injury by chemical and physical factors. It is important to note that a substance responsible for a similar ESR signal from *Brevibacteria* appeared to be a derivative of glutamic acid, thus sharing with lysodektose only a hydroxylamine group.

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