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Preparation and Properties of the Complex of Fe(III) with Peptidoglycan Monomer

MAJA TONKOVIĆ, OLGA HADŽIJA, BRANKO LADEŠIĆ, BRANIMIR KLAIĆ and SVETOZAR MUSIĆ
'Rudjer Bošković' Institute, 41001 Zagreb, P.O. Box 1016, Croatia (Yugoslavia)

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Abstract

The complex of Fe(III) with the peptidoglycan monomer (PGM) was prepared and characterised by chemical and physicochemical methods. The analyses showed that it contains two PGM molecules bonded with five Fe(III) ions. Mössbauer spectroscopy indicates that the iron oxy/hydroxy core has a dimeric or polymeric structure. Differential IR spectroscopy suggests complexation via carbonyl groups and NMR spectroscopy contribution of the carbonyl groups of D-isoglutaminyl, *meso*-diaminopimeloyl, D-alanyl residues and the carboxyl group of the C-terminal D-alanyl moiety of the peptidoglycan monomer. On the basis of the data obtained the possible structure of the Fe(III) PGM complex was proposed.

Introduction

Peptidoglycan monomer (PGM), disaccharide-pentapeptide, [2-acetamido-4-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-2-deoxy-3-*O*-(D-ethyl-1-carbonyl)-D-glucopyranose]-L-alanyl-D-isoglutaminyl-[(L)-*meso*-diaminopimeloyl-(D)-amido-(L)-D-alanyl-D-alanine] [GlcNAc-MurNAc-L-Ala-D-iGln-*meso*-(ϵNH_2)-A_2pm-D-Ala-D-Ala] was obtained by lysozyme digestion of the uncross-linked complex isolated from the culture fluid of penicillin treated *Brevibacterium divaricatum* mutant (Fig. 1) [1–5]. It was established that PGM has various biological effects. It acts as an immunostimulant and exhibits antitumor and antimetastatic activity in mice [6–8].

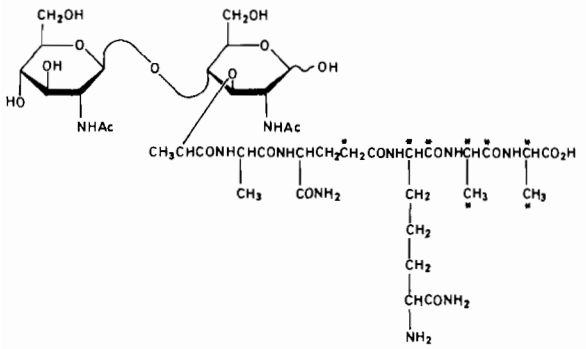

Fig. 1. Structure of the peptidoglycan monomer (PGM). ^{13}C NMR resonance lines which are missing from the spectra of the Fe(III) PGM complex are indicated by asterixes.

Many papers concerning the formation, preparation and characterisation of complexes of Fe(III) with amino acids and sugars have appeared in the literature [9–18]. PGM, as a well characterized compound, offers the opportunity to investigate the fundamental aspects of metal ion binding to small glycopeptide, but to our knowledge, such complexes with PGM have only been described with divalent cations (Cd, Cu and Zn) [19]. The ability of PGM to bind metal ions has been observed previously during its preparation [2].

In this paper we describe the preparation of the Fe(III) PGM complex, its physicochemical properties and on the basis of results obtained the tentative structure is proposed.

Materials and Methods

Method of Preparation

PGM (0.15 mmol) was dissolved in 4.5 ml of 0.1 M $Fe(NO_3)_3$ in water (0.45 mmol). The water solution was kept at room temperature for 24 h and thick oil was obtained after evaporation *in vacuo*. After addition of ethanol a pale brown amorphous precipitate was formed which was separated by centrifugation, washed six times with ethanol, once with diethyl ether and dried *in vacuo* at room temperature.

Methods of Analyses

Carbon, hydrogen, nitrogen and iron were estimated by standard microanalytical methods. Nitrates were determined spectrophotometrically. IR spectra were recorded on Perkin-Elmer spectrometer Mod. 580B. Fourier transform IR (FT-IR) spectra were recorded on a Perkin-Elmer 1710 FTIR instrument; acquisition of the spectra were performed with 10 pulses.

^{57}Fe Mössbauer spectroscopy was performed using the commercial spectrometer (Wissenschaftliche Elektronik GmbH, F.R.G.), with a $^{57}Co/Rh$ source, and the chemical shift is given with respect to metallic iron.

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^{13}C NMR spectra were recorded at 22.5 MHz on a Jeol FX 90 Q Fourier-transform spectrometer at room temperature in 5 mm o.d. tubes. The sweep width was 4700 Hz, pulse width 37 μs (90° pulse), pulse delay 1.5 s, and digital resolution 0.051 ppm/point. Chemical shifts were measured relative to that of internal 1,4-dioxane, set at 66.6 ppm downfield of that of tetramethylsilane.

Molecular weight was determined by gel filtration on Biogel P-10 column (0.9 X 56 cm) in 0.05 M acetic acid, comparing elution volumes of the complex with those of standards: cytochrome *c* (12 300), insuline (5600) and peptidoglycan dimer (2000 daltons).

Thermogravimetric analyses were performed using a Cahn RG electrobalance (U.S.A.).

Homogeneity of the Fe(III) PGM complex was established by thin layer chromatography (TLC) after visualisation of spots with reagents for sugars, amino acids, peptides and iron.

Results and Discussion

In Table 1 the results of chemical analyses and physicochemical measurements of the Fe(III) PGM complex are presented. The complex is amorphous, soluble in water giving a pale brown solution. TLC of the complex showed a spot (R_f = 0.12) which migrated differently from PGM as well as from the Fe(III) ion, containing both the glycopeptide and iron indicating the complex formation. From untreated TLC this complex was isolated and analysed by IR spectroscopy which confirmed the presence of PGM and the existence of a complex with iron.

From the thermogravimetric results (TGA) and elemental analysis the presumption of the complex composition was drawn. The molecule of the complex consists of a carbohydrate moiety, peptide chain and an inorganic part consisting of iron, nitrate ion and water. It is known [20] that when heated carbohydrates lose the first molecule of water at 120–190 °C, the second and third ones at 165–250 °C. Proteins are decomposed by thermal degradation from room temperature up to 168 °C 8.5%; 14.6% up to 240 °C and 44.3% up to 336 °C. Thermal decomposition of iron nitrate starts at 127 °C. Comparing the above values with the results of TGA it can be assumed that the first weight loss from room temperature to 134 °C (11.8%) can be attributed to losing part of the water. Significant decomposition of the complex was observed in the temperature range from 134 to 219 °C (about 40%). In that range all the water was evaporated including the water incorporated in the carbohydrate moiety, while

TABLE 1. Chemical and physico-chemical data of Fe(III) PGM complex

Method of analysis	Results	
Elemental microanalysis	calc.	found
C	32.66%	32.54%
H	6.05%	5.94%
N	10.95%	10.72%
Fe	9.50%	9.44%
NO_3	6.33%	6.58%
Thermogravimetric analysis	Weight loss at	
	RT–134 °C	11.79%
	RT–219 °C	54.42%
	RT–523 °C	86.84%
	calc.	found
Residual Fe_2O_3	13.58%	13.16%
Thin layer chromatography (TLC)	$R_{f\ PGM}$ = 0.63	
Silica gel	$R_{f\ Fe(III)}$ = 0.24	
Solvent: ethyl acetate–formic acid–water–pyridine (30:10:10:5) vol./vol.	$R_{f\ complex}$ = 0.12	
Mössbauer spectroscopy	δ = 0.366 mm/s	
	ΔE = 0.769 mm/s	
	Γ = 0.486 mm/s	
Approximative molecular weight determination	calc.	found
	2940 daltons	3500 daltons

decomposition of nitrate and peptide began. The decay of the whole complex was achieved at 523 °C, leaving the inorganic oxide (for Fe_2O_3 found 13.16%).

The ^{57}Fe Mössbauer spectrum of the complex was characterised with a symmetrical doublet at room temperature. The parameters obtained (Table 1) are similar with those obtained for Fe(III) fructose complexes [17] and other Fe(III) sugar complexes [13, 14] and indicate that Fe(III) ion is in the high spin state. In addition, these parameters support a dimeric or polymeric structure of the complex, and it seems possible that complexation takes place during the formation of a Fe(III) hydroxy polymer [21] preventing its precipitation. The presence of Fe(II) in the ^{57}Fe Mössbauer spectrum of Fe(III) PGM complex was not observed.

In the IR spectrum of the complex the bands of all the characteristic groups of PGM are present (Table 2). Furthermore, the complex structure is confirmed by the bands at 616 and 831 cm^{-1} corresponding to Fe–O and Fe–O–H stretching vibration, but the band at 831 cm^{-1}, which appeared only in the IR spectrum of the complex may be due to inorganic nitrate also [22, 23]. The differential IR spectrum (Fig. 2d) indicates that carboxyl groups are involved in the complexation (area between 1300–1600 cm^{-1}).

The ^{13}C NMR spectra of the Fe(III) PGM complex were characterised by two types of signals: resonance lines which were narrow and unshifted from their original positions and those that were noticeably broadened indicating the proximity of paramagnetic Fe(III) ion. Most of the later signals were missing from the spectra of the Fe(III) PGM complex (Fig. 3). Comparison of the chemical shift data and the spectrum of PGM [5] with the spectrum of the Fe(III) PGM complex shows that the following resonance lines are missing from the spectrum of the complex: methyl group of alanyl-5 (17.7 ppm), γ methylene group of isoglutaminyl (31.5 ppm), methyne group of alanyl-5 (49.9 ppm), α carbonyl group of *meso*-diaminopimeloyl (173.5 ppm) and carbonyl group of alanyl-4 residue (173.5 ppm). From the intensity of signals we can establish that the following signals are missing from the spectrum of the complex: methyl group of alanyl-1 or alanyl-4 residue (16.7 ppm), methyne group of alanyl-1 or alanyl-4 moiety (49.6 ppm) and one of the two methyne group of *meso*-diaminopimeloyl residue (52.8 ppm). The intensity of the signal of the β methylene group of the isoglutaminyl residue was one third smaller in the complex than in the native PGM.

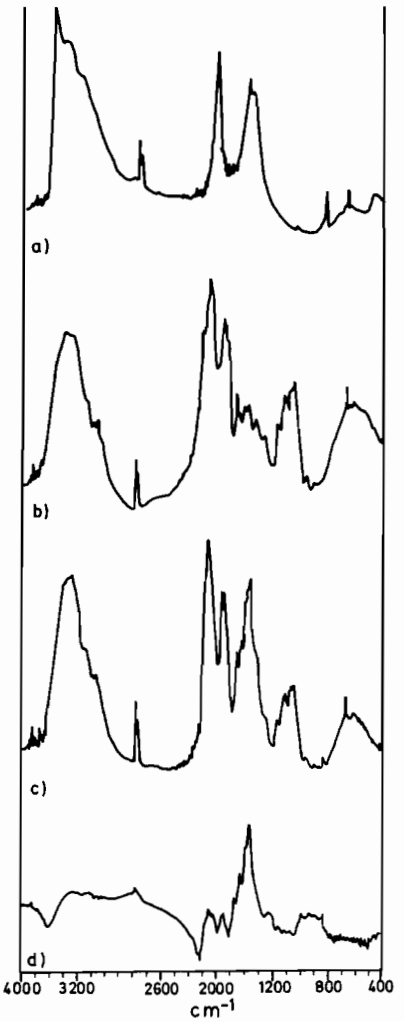

Fig. 2. FT-IR spectra of: (a) $Fe(NO_3)_3 \cdot 9H_2O$; (b) PGM; (c) Fe(III) PGM complex and (d) differential spectrum: [Fe(III) PGM] – PGM.

TABLE 2. Infrared frequencies (cm^{-1}) of PGM and the Fe(III) PGM complex

PGM	Fe(III) PGM complex	Assignment[a]
3400s,b	3305s,b	ν(O–H) assoc.; $\nu(NH_2)$
3080sh	3100sh	ν(C–H); $\nu(NH_3^+)$
2937w	2942w	ν(C–H); $\nu(CH_3)$; $\nu(CH_2)$
1684s	1684s	$\nu(COO^-)$ asymm.; δ(HOH)
1653s	1653s	$\delta(NH_2)$; $\delta(NH_3^+)$ assoc.
1559m	1541s	$\delta(CH_2)$; ν(C–O); $\delta(NH_3^+)$ sym. $\delta(NH_2)$; $\nu(COO^-)$ asymm.; ν(C–C)
1457sh	1457sh	ν(C=O); $\delta(CH_3)$; $\delta(CH_2)$
1376m	1362s	$\nu(COO^-)$; $\nu(NO_3^-)$
1320sh	1330sh	$\nu(COO^-)$ sym.; δ(C–H); $\delta(C-CH_3)$
1253sh	1260sh	δ(O–H); ν(C–O)
1163m	1162m	ν(C–O)
1049m	1147m	ν(C–O)
	831w	$\nu(NO_3^-)$; ν(Fe–O–H)
612w,b	616w,b	$\nu(COO^-)$; ν(Fe–O)

[a]Assignments were performed according to refs. 22 and 23.

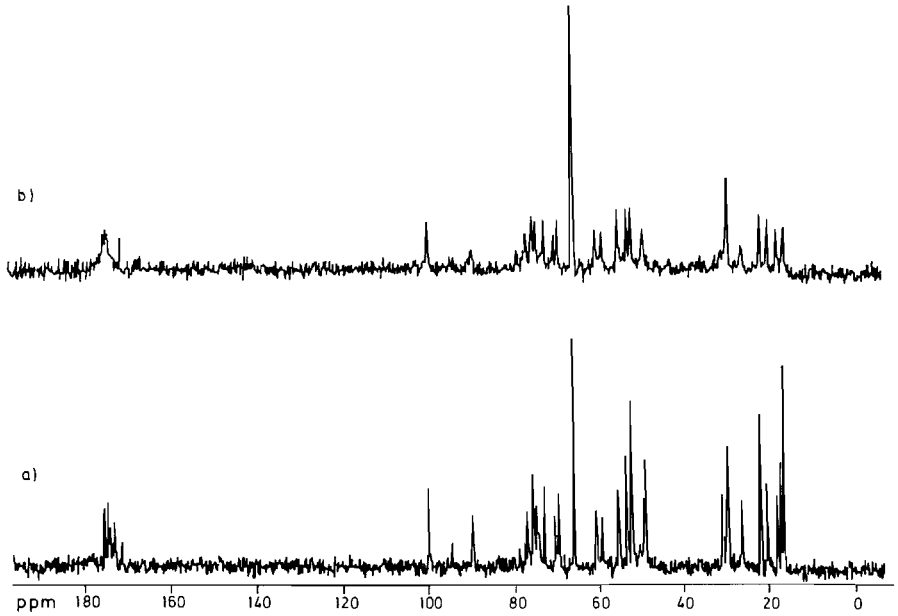

Fig. 3. The ^{13}C NMR spectra of: (a) PGM and (b) Fe(III) PGM complex.

These results indicate that the δ carbonyl group of D-isoglutaminyl, carbonyl group at the L-chiral centre of the *meso*-diaminopimeloyl residue, carbonyl group of D-alanyl (Ala-4) and carboxyl group of the C-terminal D-alanine (Ala-5) of PGM are involved in the binding of the iron ion.

On the basis of experimental data and TGA the following brutto formula of the Fe(III) PGM complex can be proposed: $Fe_5PGM_2(NO_3)_3(O)_2(OH)_6(H_2O)_{18}$ (Table 1).

In addition to the above analysis the molecular weight of approx. 3500 daltons obtained by gel filtration supports the assumed composition of the Fe(III) PGM complex containing two PGM bonded with five Fe(III) ions (Table 1).

It is known that complexation of iron ions with organic ligands is accomplished through the iron-oxy/hydroxy core [14, 17, 21, 24, 25]. So, it seems possible that PGM forms a complex with an iron-oxy/hydroxy core in a similar way. On the basis of the experimental data obtained we propose that the Fe(III) PGM complex has a structure as presented in Fig. 4.

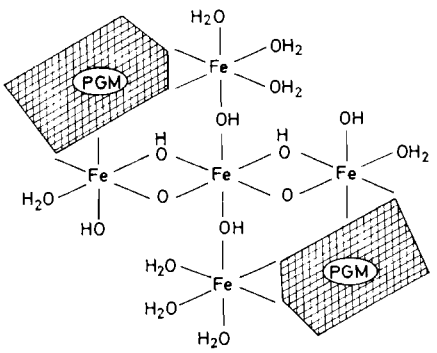

Fig. 4. Proposed structure of the Fe(III) PGM complex.

Acknowledgements

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