THE STAPHYLOCOCCAL PEP-DEPENDENT PHOSPHOTRANSFERASE SYSTEM COMPLETE ASSIGNMENT OF THE AROMATIC ^{1}H RESONANCES OF THE PHOSPHOCARRIER PROTEIN HPr

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Introduction

The phosphocarrier protein HPr is part of the bacterial PEP-dependent phosphotransferase system which catalyses vectorial phosphorylation of carbohydrates (1, 2).
The following features make this protein an ideal candidate for NMR research:

1. The molecular weight is sufficiently small to obtain well-resolved spectra.
2. The protein contains only 5 aromatic residues: 1 Phe, 1 His and 3 Tyr resulting in a rather simple aromatic region of the NMR spectrum (3).
3. The amino acid sequence as well as the prediction of the secondary structure of the molecule are available (4).
4. The histidine residue 15 is the active center of the protein carrying the phospho-group at Nitrogen 1 of the imidazole ring during the phosphotransfer reaction (5).

In this paper we describe the assignment of the tyrosine resonances after nitration of the tyrosines with tetranitromethane (TNM) followed by isolation of tryptic nitrotyrosyl peptides, which allow proper positioning of the nitrotyrosyl residues within the sequence. Together with the chemical characterization of the nitrotyrosyl derivatives, the spectral change occurring by the introduction of the nitrogroups was recorded, which resulted in the assignment of the tyrosine resonances.

Results and Discussion

Nitration of HPr

Nitration of HPr was performed according to described methods (6) with a tenfold excess of TNM per tyrosine at pH 8.5 in Tris-HCl buffer, the product $(\text{nitrotyrosyl})_1$ HPr was formed preferentially. Increased reaction time and a greater excess of TNM led to the formation of $(\text{nitrotyrosyl})_2$ HPr. $(\text{Nitrotyrosyl})_3$ HPr could only be obtained using $NaHCO_3$ buffer pH 8.5, as found out empirically. The nitrotyrosyl derivatives could be separated from each other by ion exchange chromatography on DE52 cellulose.

Assignment of the Tyrosine Resonances

Spectra run on the purified derivatives revealed that in $(\text{nitrotyrosyl})_1$ HPr Tyr A (pK 10.5), in $(\text{nitrotyrosyl})_2$ HPr Tyr A+B (pK 11.5), in $(\text{nitrotyrosyl})_3$ HPr all AA'BB' signal patterns of the tyrosines were modified to the expected ABC pattern of 3-nitrotyrosine (Fig. 1).

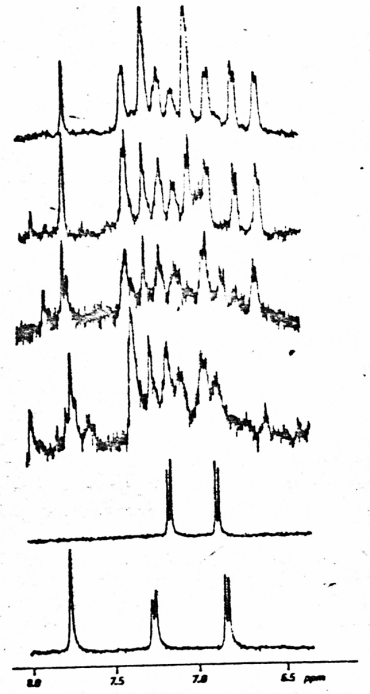

Fig. 1 Aromatic Regions of HPr and its Nitrotyrosylderivatives at 360 MHz from Top to Bottom
HPr
$(\text{Nitrotyrosyl})_1$ HPr
$(\text{Nitrotyrosyl})_2$ HPr
$(\text{Nitrotyrosyl})_3$ HPr
free Tyrosine
free Nitrotyrosine
The protein concentration of the (nitrotyrosyl) HPr derivatives was 2-4 mg/ml. 1000 transients were accumulated and Fourier transformed

Finger printing, isolation and characterization of the tryptic nitrotyrosine containing peptides, matching the peptides with the known amino acid sequence led to the assignment of all aromatic protons of HPr as indicated in Fig. 2.

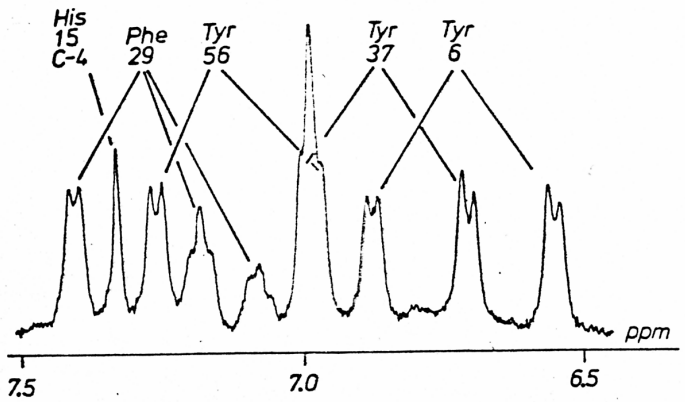

Fig. 2 Assignment of all aromatic resonances of HPr. The Histidin C2 signal is not shown.

To our knowledge HPr is the first protein studied by NMR where a total assignment of the aromatic region has been accomplished by the combination between NMR and chemical methods without the knowledge of the crystal structure.

Interactions detectable by NMR between Nitrotyrosyl and other Amino Acid Residues

The introduction of the nitrogroup into the tyrosyl residues creates a different electronic structure of this residue. The most obvious feature is the low pK of nitrotyrosine (∿7.1) compared to tyrosine in HPr (≥10.5). Therefore the titration of nitrotyrosyl residues can be performed far below the pH where the protein starts to denature.

Upon titrating the various nitrotyrosyl HPr derivatives the following interactions between nitrotyrosyl and other residues were observed:

1. His 15, the active center of the protein, cotitrates with nitrotyrosine 56; His 15 is postulated to be on the outer surface of the protein according to the structural prediction. The NMR titration and chemical shift data are in agreement with this prediction. Tyr 56 is also located at the surface of HPr; the evidence is manyfold: The NMR titration behaviour and chemical shift are close to the data for the free tyrosine, Tyr 56 is the residue which is nitrated preferentially.
2. One methionine residue strongly cotitrates with nitrotyrosine 37 (Fig. 3).

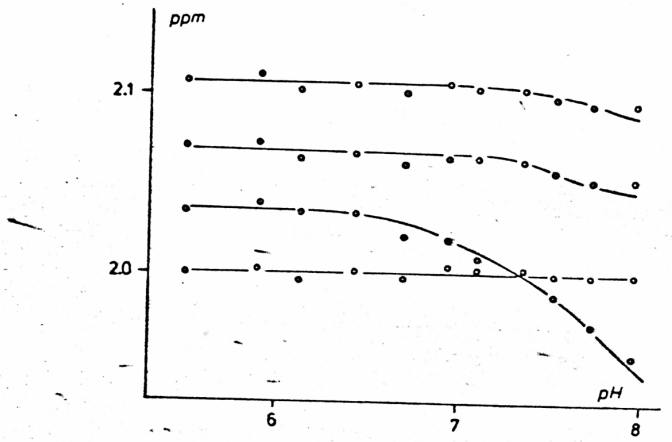

Fig. 3 Titration Curves of the Methionine Resonances in (Nitrotyrosyl)$_2$HPr

3. The high field signal at -0.18 ppm assigned to a valine residue is abolished in the (nitrotyrosyl)$_3$HPr derivative (Fig. 4).

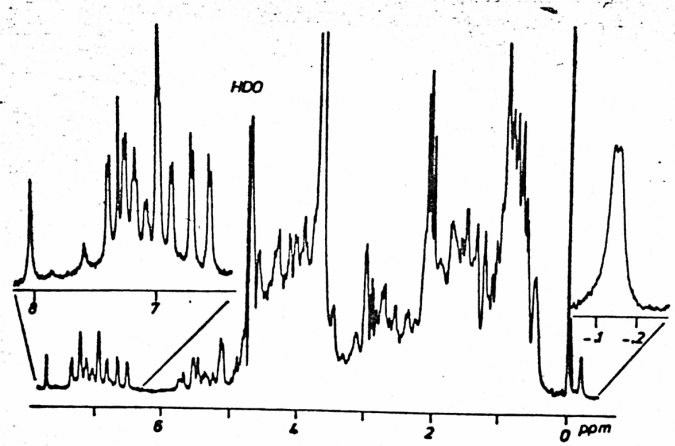

Fig. 4 Complete ^{1}H NMR Spectrum of HPr at 360 MHz. The insert at the right side represents the doublet shifted to high field, assigned to a valine resi-

due. The signals at 2 ppm represent four methionine residues.

The structural features of the protein deduced from the interacting residues are summarized in Fig. 5

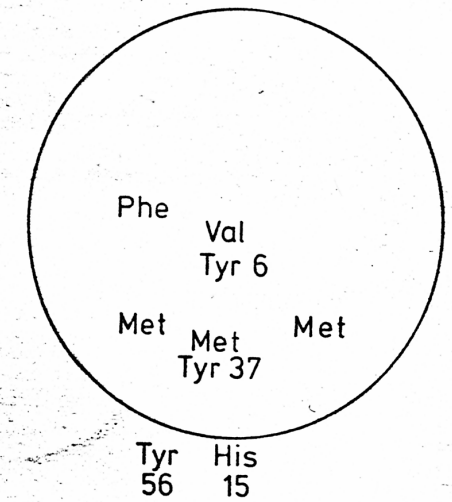

Fig. 5 Symbolic Drawing of the Spectral Relations between several Amino Acid Residues as derived from the NMR Measurements. The circle represents the surface of the protein.

which represents a speculative model of how certain residues are located within the protein molecule.

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