

## ARTICLES

# **The phosphoenolpyruvate-dependent phosphotransferase system of *Staphylococcus aureus*. Complete tyrosine assignments in the <sup>1</sup>H nuclear- magnetic-resonance spectrum of the phosphocarrier protein HPr**

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Upon nitration of the phosphocarrier protein HPr three nitrated derivatives of the protein were isolated: mononitrated HPr, dinitrated HPr and trinitrated HPr. Tryptic digestion of the derivatives leads to nitrotyrosine-containing peptides which were isolated and characterized by amino acid analysis. This resulted in the determination of the positions of the nitrated tyrosyl residues in the amino acid sequence. In mononitrated HPr only Tyr-56 was modified, in dinitrated HPr both Tyr-56 and Tyr-37 had reacted with the nitrating agent; modification of all three tyrosyl residues in trinitrated HPr required more drastic reaction conditions. The nuclear magnetic resonance spectra of the three derivatives allowed the assignments of the tyrosine resonances as follows: Tyr-A and Tyr-B with pK values of 10.5 and 11.5 were designated Tyr-56 and Tyr-37 whereas Tyr-C, whose protons are not titratable before denaturation of the protein, was assigned to Tyr-6 in the amino acid sequence. The nitration studies, together with the titration behaviour of the three tyrosines, indicate the topology of the tyrosyl residues to be as follows: Tyr-56 is located at the surface, Tyr-37 is slightly buried, Tyr-6 is deeply buried. The nitrotyrosyl derivatives retain their biological activity.