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Conjugation Reaction of N-Acetyl Tyrosine with Adenosine Triphosphate (ATP) Catalyzed by Fe(II)/H_2O_2 System

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Short Communication

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ABSTRACT

A conjugation reaction of N-acetyl tyrosine with adenosine triphosphate (ATP) was investigated by mass spectrometry in order to understand the mechanism to tyrosine phosphorylation in proteins. A reacting solution containing 10 mM of N-acetyl tyrosine, 6.4 mM of ATP disodium, 250 μM $FeCl_2$, and 0.15% H_2O_2 was prepared in 500 μL of 20 mM ammonium bicarbonate (pH 7.4). The reactions were allowed to proceed for 48 h at room temperature followed by mass spectral analysis. It was found that this reaction was catalyzed by the Fe(II)/H_2O_2 system and the carboxyl terminus of N-acetyl tyrosine that bound to the ferrous ion. The reaction products seem to be the condensates of the phenolic group of tyrosine and the phosphate groups of ATP at a stoichiometry of 1:1 and higher which correspond to an m/z value of 736, 981 and 1248. The study also indicates that an additional step of ATP hydrolysis is needed to produce Phosphor-N-acetyl tyrosine (MW 303) from the conjugate. This reaction may be the precursor step of tyrosine phosphorylation in live organisms.

INTRODUCTION

Tyrosine phosphorylation is a reaction of adding a phosphate (PO_4^{3-}) group of adenosine triphosphate (ATP) to tyrosine residues in proteins. This reaction was found in 1979 in a study of the multi tumor virus, and later tyrosine phosphorylation was considered as a key step in protein signal transduction and regulation of enzymatic activity [1-3]. The reaction of the transfer of the phosphate group must occur through the catalysis of tyrosine kinase. Without the enzyme, this reaction can happen only under harsh conditions of organic synthesis [2,4,5] that do not exist in the body. The specific mechanism of the enzymatic catalysis is not very clear. I hypothesize that the reaction may be related to transition metal ions such as Fe(II) and Cu(II) because these ions can produce free radicals, which then react with many species in biological systems [6-8].

The author's previous research has shown that the rate of metal-catalyzed oxidation is significantly increased when tyrosine is adjacent to a carboxyl group as in Glu-Tyr and N-acetyl tyrosine. This is due to the binding of the carboxyl group with iron and copper ions which produce radicals near the phenolic group of tyrosine [6]. On the contrary, the N-terminus of tyrosine and other positively charged groups of peptides slows the oxidation reaction because they disfavor the binding of the metal ions. The oxidation products of Glu-Tyr and N-acetyl tyrosine were also more complicated than the one of tyrosine which was primarily monohydroxylated tyrosine (or DOPA) [6-9]. For example, two of the oxidation products of N-acetyl tyrosine are quinone like compounds of oxidized DOPA. Some of these oxidation products may be reactive and subject to further chemical modifications. In a separate study, the author found that acetylsalicylic acid (aspirin) could form a three-component complex with ferrous ion and an endogenous substance, gluconic acid, and this complexation accelerates the hydrolysis of aspirin. Moreover, the hydrolysis products, salicylic acid and acetic acid, further reacted with the hydroxyl groups of gluconic acid to form salicylate-gluconic acid conjugates and acetylated gluconic acid via a condensation reaction [10]. It is known that the phosphate group of ATP can complex with metal ion [11], hence it is likely that tyrosine, ATP and transition metal ions undergo similar mechanism by forming a tertiary complex intermediate to produce phosphotyrosine. The following research is trying to demonstrate the possibility of this reaction path.

EXPERIMENTAL PROCEDURES

A reacting solution containing 10 mM of N-acetyl tyrosine, 6.4 mM of ATP disodium, 250 µM $FeCl_2$, and 0.15% H_2O_2 was prepared in 500 µL of 20 mM ammonium bicarbonate (pH 7.4). These chemicals were purchased from Sigma Aldrich. The reactions were allowed to proceed for 48 hours at room temperature. Samples were then diluted by a ratio of 1:5 in 80% methanol/1% formic acid for mass spectral analysis (Orbitrap, Thermo Scientific) and the instrument parameters are shown as follows: Ion max ESI was used; selected Ion Monitoring (SIM) FTMS data were acquired by direct infusion at 3 uL/min; AGC=5 e4; resolution=60 K; 1 microscan; max inject time of 50 ms; spray voltage 4.3 kV; tube lens=110 V.

RESULTS AND DISCUSSION

The mass spectrum of N-acetyl tyrosine is illustrated in **Figure 1a.** The peak of 224 m/z was the molecular ion and the peaks of 246 m/z and 268 m/z were the sodium adducts of N-acetyl tyrosine. The intensity of the peak of 224 m/z decreased by about 44% after the reaction. The mass spectrum of ATP is shown in **Figure 1b.** The molecular ion peak of ATP (507 m/z) was not quite visible while peaks of 530 m/z and 552 m/z were detected which correspond to the mono- and di-sodium salts of ATP. The intensity of these two peaks decreased by about 66% and 50% respectively after the reaction. The peaks of 491 m/z and 513 m/z may be the ion and the sodium adduct of deoxy-ATP or the sodium adducts of di-acetyl tyrosine; they are not present in the spectrum of pure ATP [12]. The mass spectra of the reaction products are displayed in **Figure 1c**. The peak of 736 m/z and 758 m/z can be attributed to the formation of the condensate of N-acetyl tyrosine and ATP as shown in **Scheme 1.** This molecule has a molecular weight of 712 Da that can associate with one or two sodium ions to become 735 Da and 758 Da. There were other peaks above 1000 w/z which are related to structures of other conjugation products of N-acetyl tyrosine and ATP (e.g., at 2:1 and 3:1 stoichiometry) as this reaction is continuous and not quite selective. Interestingly, only very small amount of phospho-N-acetyl tyrosine (303 m/z and 304 m/z) was detected in this experiment which is equivalent to 1/44 of that of N-acetyl tyrosine (Spectrum shown in **Figure 1d**). This suggests that the phosphate groups of ATP did not hydrolyze, which would require enzymatic cleavage in biological systems.

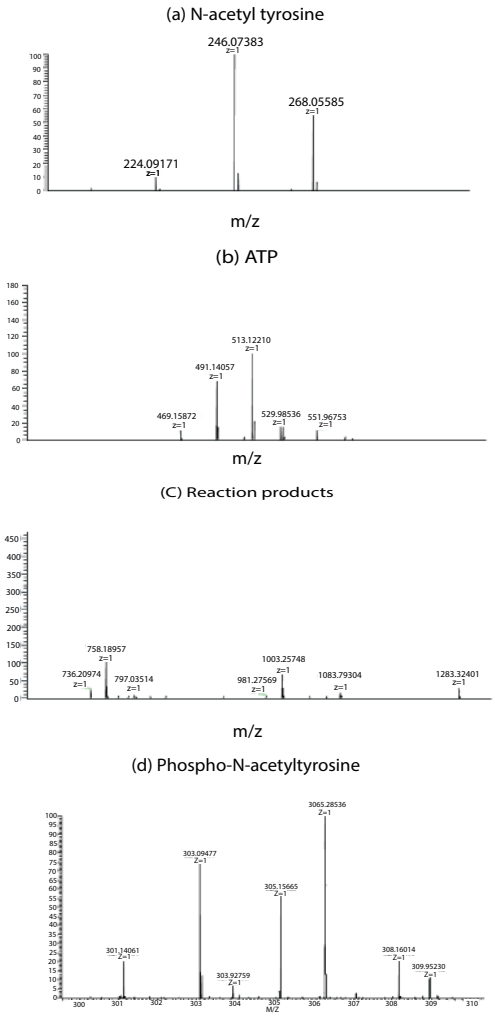
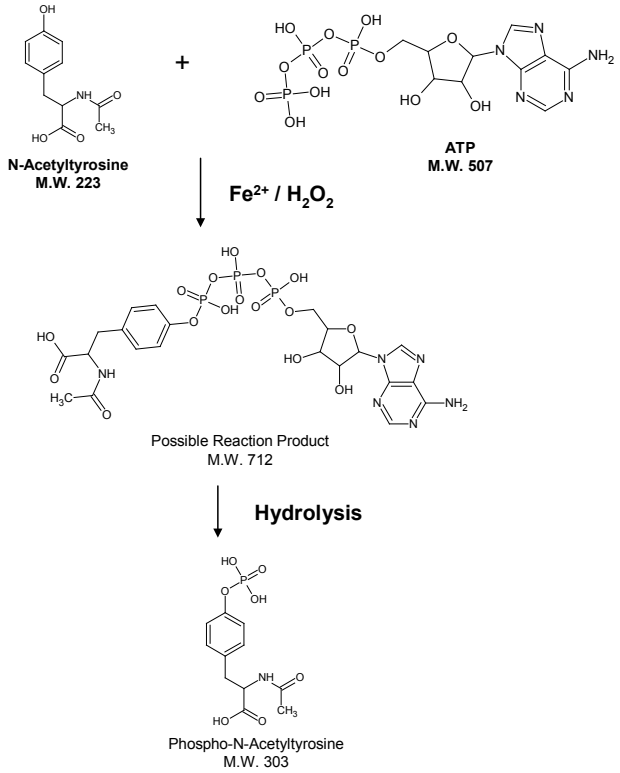


Figure 1. Mass spectra of the reacting solution containing 10 mM of N-acetyl tyrosine, 6.4 mM of ATP disodium, 250 µM $FeCl_2$, and 0.15% H_2O_2 (diluted by 5 times for analysis).



Scheme 1. Reaction of N-Acetyl tyrosine with ATP

The results of this research infers that tyrosine phosphorylation in biological systems may be an oxidation reaction analogous to ATP synthesis [13,14], and that the tyrosyl residue may be directly bound to or located very closed to an Fe(II) moiety in tyrosine kinase to catalyze the reaction. It can also get the electrons from the electron transport chain which contains transition metal ions in the form of Fe-S clusters and multiple metal cofactors [15-18]. The enzyme also has an additional function that hydrolyzes the phosphate groups off from ATP subsequent to the oxidative phosphorylation reaction.

CONCLUSION

N-Acetyl tyrosine and adenosine Triphosphate (ATP) can couple together in the presence of Fe(II)/ H_2O_2 through a condensation reaction. This reaction may be the precursor step of tyrosine phosphorylation in live organisms. It may also have other implications to the pharmacology and toxicology of N-acetyl tyrosine which is used as a common brain supplement.

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