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The Self-catalytic Esterification Reaction of *O*-Phosphoryl Serine Derivative

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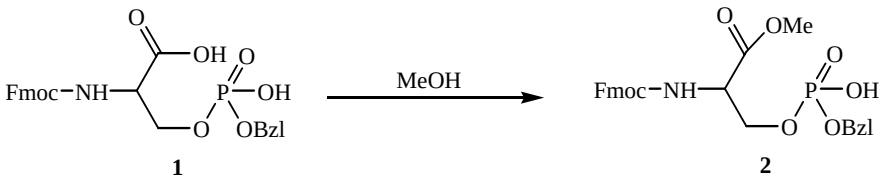
Abstract: *O*-Phosphoryl serine derivative can perform self-catalytic esterification reaction in the mixture of CH_3OH and $CHCl_3$ at the room temperature. The phosphoryl group participation was the key step of the esterification. This type of reactions were proposed through an intermediate of mixed phosphoric-carboxylic anhydride that might provide a clue to the function of the phosphoryl group in the phosphorylated enzymes and in the prebiotic synthesis of protein.

Keywords: *O*-Phosphoryl serine, self esterification, phosphoryl group participation.

Phosphoryl group plays a crucial role in life chemistry, not only because of its high content in nucleic acids, but also due to its regulative effect on enzyme activities. It is even worthwhile to note that during protein biosynthesis, phosphorylation of amino acid is the key reaction. Formation of high-coordinate phosphoric intermediate is usually described as a key step in most enzyme catalytic mechanisms[1~3]. Hence, it would be critical to understand the intrinsic relationship between the phosphoryl group and the amino acid residues during protein phosphorylation, especially the formation of high-coordinate phosphoric intermediates.

Here we report that *O*-phosphorylated serine derivative **1** can perform self-catalytic esterification in the mixture of CH_3OH and $CHCl_3$ at the room temperature (**Scheme 1**). An intramolecular mixed phosphoric-carboxylic anhydride, was proposed as the intermediate for the reaction[4~6].

Scheme 1 The esterification of *O*-phosphorylated serine derivative **1**



1 **2**

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Figure 1 ESI-MS/MS negative spectrum of compound **1** (A) and the product **2** (B)

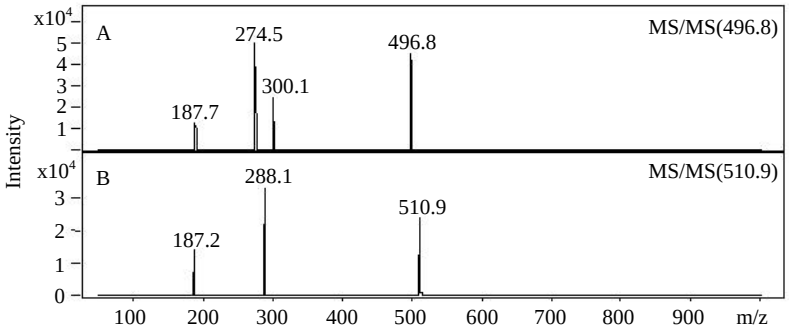
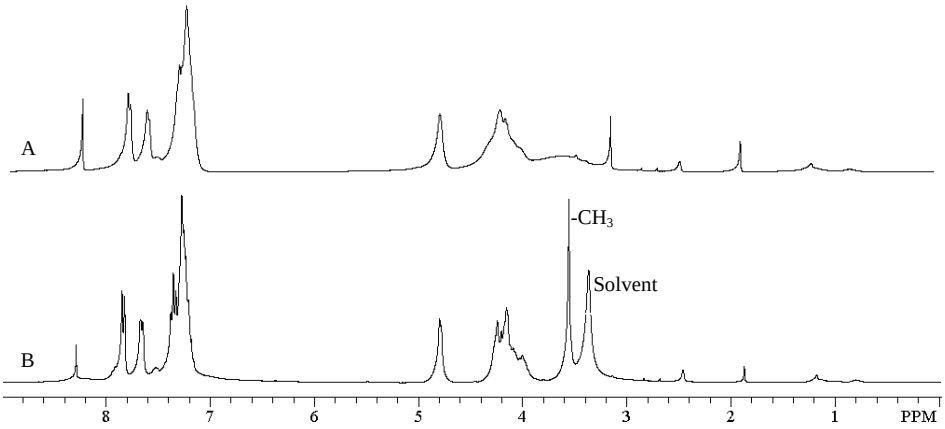


Figure 2 The ^{1}H NMR spectra of compound **1**(A) and compound **2**(B)



O-(Benzyloxyhydroxyphosphoryloxy)-N-Fmoc serine **1** was prepared according to reference[7]. The mass spectra were obtained using a Bruker ESQUIRE-LC^{TM} ESI ion trap spectrometer equipped with a gas nebulizer probe. The product and **1** were analyzed by multi-stage ESI mass spectrometry (MS^n). The ^{31}P NMR spectra were taken on a Bruker ACP 200MHz spectrometer with 85% phosphoric acid as the external reference. The ^{1}H and ^{13}C spectra were recorded on a Joel 300 NMR spectrometer in DMSO solvent.

When **1** was kept in methanol for several days, a new peak at *m/z* 510.9 corresponding to **1**+14 was detected in ESI-MS mass spectra. In its tandem spectra, the mass of the corresponding fragment ion (288.1) was also added by 14 (**Figure 1**).

From the ^{1}H NMR spectrum, we can find that there is a new sharp peak at 3.56 ppm corresponding to methyl protons of $COOCH_3$ (**Figure 2**). There is also a new peak at 51 ppm in the ^{13}C NMR spectrum, and according to the DEPT spectrum, it is an odd carbon atom. From these results, we can propose that there is a methyl group in compound **2.** Furthermore, ^{31}P NMR was used to compare the chemical shifts of the product **2** and compound **1**, both of which are about –3.0 ppm in DMSO. The fact, no

much change of the chemical shift in ^{31}P spectrum also proved that the methyl group is bonding to the carboxylic carbon but not to the phosphorus atom. Therefore, the new compound **2** is *O*-(benzyloxyhydroxyphosphoryloxy)-N-Fmoc serine methyl ester. If the time of incubation was prolonged to two weeks, the yield of **2** would be 60%.

In addition, some other experiments were carried out to study if the compounds without phosphoryl group also had the same property. Some similar compounds, such as Fmoc-Ser-OH, Fmoc-Ser(O-^{t}Bu)-OH were chosen. After mixed with CH_3OH and $CHCl_3$ at the room temperature for a long time, no similar reaction was detected according to the ESI-MS. Moreover, when the compound Boc-Ser(PO_3Pen_2H)-OH was used in the same condition as others, this type of reaction was not observed, probably due to the two cyclopentyl (Pen) protecting groups in the phosphoryl group. According to the ESI mass spectrum, *O*-(Benzyloxyhydroxyphosphoryloxy)-N-Fmoc serine **1** can not perform self-catalytic esterification reaction in the mixture of ethanol, propanol, or isopropanol at the room temperature.

Considering our previous work on N-phosphoryl amino acids[6], it seemed that the reaction was attributable to the presence of a phosphoryl group. The phosphoryl group in the *O*-phosphoryl serine activated itself to carry out the above reaction.

In the self-activation reaction, may exist the intermediate of mixed phosphoric carboxylic anhydride in which phosphoryl group and carboxylic group activate each other. The nucleophile OCH_3 could attack the carboxylic carbon to give the ester **2**.

In conclusion, *O*-phosphoryl serine was able to perform esterification self-activated by the phosphoryl group. The reaction might through the intermediate of mixed phosphoric-carboxylic anhydride. In the real enzyme, a three-dimensional conformation might promote to form such intermediate and initiate the biochemical process under mild condition. Consequently, the phosphoryl group participation plays a key role to the chemistry of phosphorylated enzymes.

Acknowledgments

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