On the Mechanism of the Cation- and Substrate Analog-activated Enzyme Reaction: Glucosamine-6-phosphate Deaminase

We present the case of glucosamine-6-phosphate deaminase reaction in which the production of fructose-6-phosphate(p) occurs in the absence and presence of divalent cations such as Mn^{++}, Hg^{++} and Co^{++} and the substrate analogue (A), N-acetyl glucosamine-6-phosphate, respectively, but with the maximum rate in the presence of both the cation and the analogue[1]. The rate of the reaction can be expressed as follows:

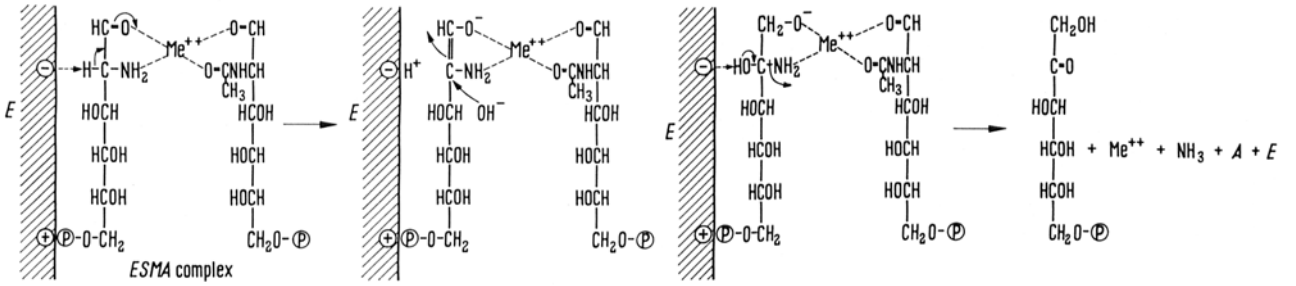
$$dp/dt = k(ES) + k'(ESM) + k''(ESMA)$$

where k's are rate constants, and (ES), (ESM) and ($ESMA$) are the enzyme-substrate, enzyme-substrate-metal and enzyme-substrate-metal-analogue complexes, respectively.

From the pH profile of the reaction with the maxima at pH 8[1] and 8.5[2,3], a possible mechanism of the reaction can be suggested, accounting for the overall reaction in the presence of both the cations and substrate analogue.

The first step may be the formation of a chelate between ES complex and the analogue. Then, a basic group with high pK predictable from the pH profile may deprotonate carbon 2 of the substrate bound, followed by the shift of the pair of electrons towards the carbonyl-metal bond. The resulting enolate[4] will subsequently be hydrated and deamination assisted by the chelation will follow, as shown below:

where E represents the enzyme molecule.



It appears that the essential feature of the mechanism suggested here is the enhancement of the rate by the chelation between ES complex and the latter's in the presence of the cations and substrate analogue. It is possible that both the substrate and analogue are bound to the enzyme surface to provide rigidity for favourable chelation. The mechanism proposed can be of value in understanding the activation of enzyme reactions[5].

Zusammenfassung. Die Reaktion der Glukosamin-6-Phosphat-Deaminase, an der Metallionen und ein Substratanalogon teilnehmen, wurde vom mechanistischen Standpunkt aus bearbeitet und auf der Basis der Komplexierung des ES-Komplexes mit den obengenannten Aktivatoren dargestellt.

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4 The fact that a significant amount of enol was formed in the alkaline solution of 2-amino-D-glucose supports existence of such an enolate intermediate in the enzyme reaction (P.-S. Song and C. O. Chichester, unpublished work).
5 *Acknowledgment.* The author expresses his appreciation to the Office of Atomic Energy, Korea, for financial support.
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Red Cell Agglutination by S Protein, a Lipoprotein from Erythrocyte Stroma

It was previously reported by de C. Baker[1] that homologous and some heterologous red cells were agglutinated by haemolysates made from rabbit, rat and guinea-pig red cells. Evidence was also available that this phenomenon was probably due to a substance liberated from the stroma, rather than to haemoglobin, since the filtered haemoglobin produced no agglutination of intact red cells.

The following study was undertaken to investigate this possibility. Therefore, the protein now known as S protein, was separated from the cells of man and of a number of different animals by the original method of Moskowitz et al.[2]. Isotonicity was then restored by dialysis against large quantities of buffered NaCl in the cold for 48 h. Final centrifugation was always necessary for elimination of some residual turbidity. The NaCl-buffer recommended by Ponder[3] for the red cells, is made by mixing 75 ml of

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