## 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:

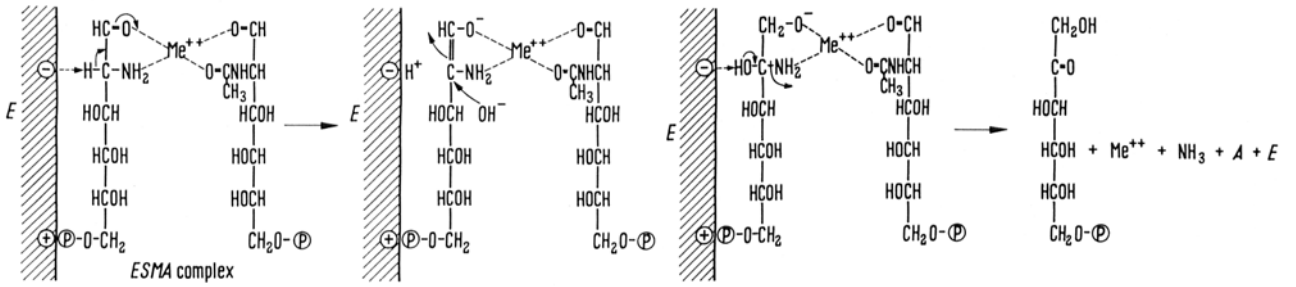
$$dp/dt = k(ES) + k'(ESM) + k''(ESMA)$$

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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1 T. N. S. Varma and B. K. Bachhawat, Biochim. biophys. Acta *69*, 464 (1963).
2 D. G. Comb and S. Roseman, J. biol. Chem. *232*, 807 (1958).
3 T. N. Pattabiraman and B. K. Bachhawat, Biochim. biophys. Acta *54*, 273 (1961).
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

1 S. B. de C. Baker, Nature *185*, 547 (1960).
2 M. Moskowitz, W. B. Dandliker, M. Calvin, and R. S. Evans, J. Immunol. *65*, 383 (1950).
3 E. Ponder, *Hemolysis and Related Phenomena* (Grune & Stratton, New York 1948), p. 104.

1.2 g/100 ml NaCl with 25 ml of a mixed buffer composed of 72 ml of $M/15$ $Na_2HPO_4$ and 28 ml of $M/15$ $KH_2PO_4$. The pH was 7.0 at 25°C. All samples of S protein were analysed for nitrogen by micro-Kjeldahl method; thereafter, they were brought to the same nitrogen content (mg 130%). The presence of haemoglobin in the solution of S protein should not interfere with the present results, since haemoglobin, as established before, does not cause haemagglutination.

The red blood cells were washed, before using, five times with 0.9% NaCl solution and made up to a 3% suspension in NaCl-buffer. The reaction was carried out on microscope slides. A large drop of cell suspension was added to each drop of S protein. Complete mixing was secured first by stirring, later by rocking to and fro. The progress of reaction was observed against a white background. The test is observed for 15, or at the most 30 min. Fine agglutinates were graded as one plus, medium-size as two plus, and large clumps as three plus.

As shown in Table I, S protein was active in producing agglutination of rabbit, rat and guinea-pig erythrocytes. However, in contrast to the above-mentioned studies, this haemagglutinating activity was not confined to the three rodent species here considered but was also found in the samples from horse blood. Moreover, guinea-pig S protein acted only upon the red cells of the same species whereas the human one failed to cause agglutination of cells of any species.

The explanation of these findings is not easy; it seems, however, reasonable to regard them as really due to S protein and not due to heterogenetic antibodies derived from residual plasma in the protein. Table II indicates, in fact, that there was no relationship between its haemagglutinating property and the cross reactions observed.

Table I. Haemagglutinating activity of S protein derived from man, horse, rabbit, guinea-pig and rat on the corresponding red cells

| Red cells from: | S protein from red cells of: | | | | |
|---|---|---|---|---|---|
| | Man[a] | Horse | Rabbit | Guinea-pig | Rat |
| Man | 0 | 0 | 0 | 0 | 0 |
| Horse | 0 | 0 | 0 | 0 | 0 |
| Rabbit | 0 | ++ | 0 | 0 | 0 |
| Guinea-pig | 0 | ++ | ++ | +++ | +++ |
| Rat | 0 | +++ | 0 | 0 | 0 |

[a] Human S protein was obtained from 0 Rh positive red cells of normal donors.

Table II. The lack of relationship between red cell agglutination by S protein and heterogenetic antibodies derived from residual plasma proteins

| Red cells from: | Plasma of: | | | | |
|---|---|---|---|---|---|
| | Man | Horse | Rabbit | Guinea-pig | Rat |
| Man | 0 | 0 | +++ | 0 | 0 |
| Horse | 0 | 0 | 0 | 0 | 0 |
| Rabbit | +++ | 0 | 0 | + | 0 |
| Guinea-pig | +++ | 0 | ++ | 0 | 0 |
| Rat | +++ | 0 | ++ | +++ | 0 |

*Riassunto.* La proteina «S» dello stroma eritrocitario di alcune specie animali (uomo, cavallo, coniglio, cavia, ratto) si è dimostrata capace, per alcune di esse, di agglutinare le emazie omologhe ed eterologhe. Il fenomeno sembra legato alla proteina «S» e non ad anticorpi eterogenetici derivanti da tracce di plasma presenti nella proteina stromale.

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## Androgen Influence on Protein and Enzyme Pattern of Rat Kidney[1]

Column chromatography on substituted cellulose ion exchanger has been used in previous researches to fractionate soluble proteins extracted from the submaxillary gland of male and of female mouse. Clear-cut differences in the protein composition of male and female glands were demonstrated, the chromatographic patterns appearing distinctive and characteristic for each sex[2].

By using column chromatography BOND was able to demonstrate a sex-specific protein, of still unknown function, in rat liver[3]. This protein is present in the male rat liver and is susceptible to hormonal manipulation. We have extended this type of investigation to other organs known for their sexual dimorphism. The present paper is concerned with chromatographic fractionation of proteins of extracts of male and female rat kidneys; four enzyme activities have been localized in the fractions; in addition the effect of testosterone treatment on the female pattern has been studied.

*Materials and Methods.* Adult albino rats of an inbred laboratory strain were used. The rats were kept housed in the same animal room and fed the same diet *ad libitum*; water was given freely. Treated female rats received 3 mg of testosterone propionate injected into the gluteal muscle every other day for a total of five injections (10-day course) in one group (6 animals), 12 injections (24-day course) in the other (6 animals). Control female rats received no injections. 48 h following the last dose of testosterone, the animals were killed by decapitation. The kidneys were removed and homogenized in 4 volumes of tris-(hydroxymethylaminomethane)-phosphate buffer 0.005 $M$, pH 7.25 in a Waring Blendor at 0–3°C, for 2 min. The homogenate was centrifuged at 0°C at 20,000 × $g$ for 30 min. The supernatant was decanted and dialysed for 18 h against

[1] Supported by NIH Grant and by the CNR.

[2] R. LEVI-MONTALCINI and P. U. ANGELETTI, International Symposium on Salivary Gland, Seattle (1962).

[3] H. E. BOND, Nature *196*, 242 (1962).