

Enzyme action: The delineation of novel strategies based on reaction mechanisms and transition states

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Abstract - Novel synthetic strategies based on the chemical models designed to simulate the enzyme action in some important biological processes have been developed. Successful chemical simulation of some vital cyclic operations of Nature, for example, ATP-Imidazole cycle - wherein a daughter imidazole is grown on a parent imidazole template via a cyclic pathway that is linked to the biosynthesis of the purine code bases ATP and GTP as well as to the imidazole amino acid histidine - and urea cycle wherein ornithine, a non-coded amino acid is used as a carrier molecule for urea via cyclic pathway that is linked to the biosynthesis of arginine - has provided a novel concept of the use of templates and carrier molecules in organic synthesis. A practical methodology for the synthesis of C-terminal amides from serine and threonine extended polypeptide precursors has been designed, patterned on the mechanism of action of pituitary enzymes for the generation of bioactive C-terminal polypeptide hormones from their glycine extended precursors.

An aspect of enzyme action that has hitherto received scant attention is that in a large number of cases the reaction so mediated brings about changes remarkably close to those carried out with model systems under the laboratory conditions. Our endeavours at the chemical simulation of highly evolved and vital biological processes envisaged, *inter alia*, a correlation of processes mediated by enzymes on the one hand and those by using purely chemical means on the other. The long range objective of such study is to bring about a symbiosis of both enzymes and chemically mediated reactions to make the synthesis of complex target molecules effective. The work reported here would show that it is possible to bring about enzyme mediated changes effectively by chemical methodologies. The importance of this lies in the fact that chemically mediated processes are flexible in terms of substrates, reaction conditions and isolation procedures. We have also successfully demonstrated that the proper delineation of chemical means to bring about the enzyme mediated processes can be taken advantage of to generate novel strategies in organic synthesis for the preparation of substrates of biological importance.

From this vantage, we have examined three carefully chosen key biological transformations, namely, the chemical simulation of the ATP-Imidazole cycle, the Urea cycle and α -amidating action of pituitary enzymes.

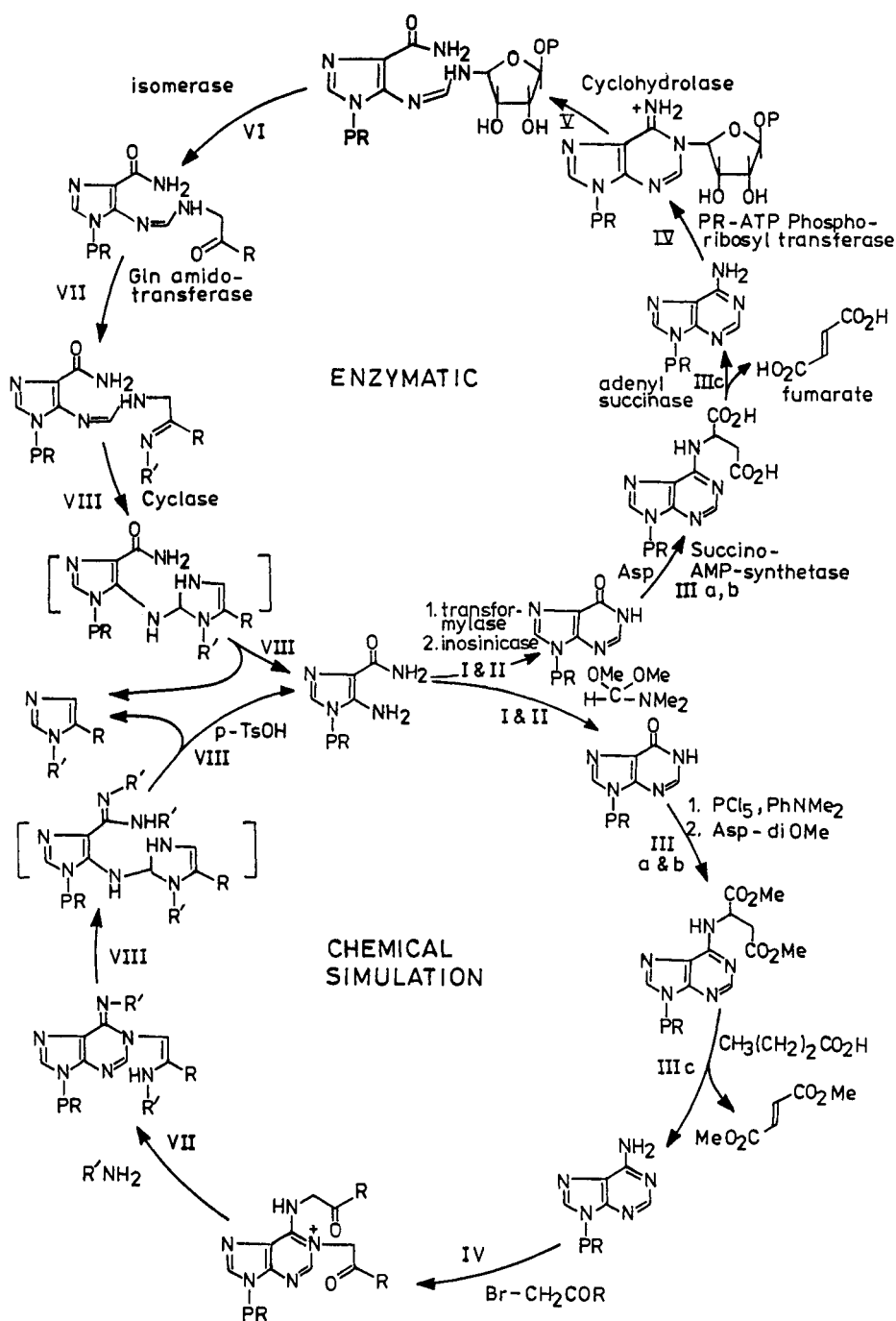
A comparison of the ATP-Imidazole cycle in terms of *in vivo* (ref.1) and laboratory simulation operations (ref.2) is presented in Figure I. It could be seen that the chemical simulation tantamounts to accomplishing 8 enzyme mediated reactions. The first two steps of the operation leading to hypoxanthine involving formylation and cyclization of the parent template, not requiring selectivity, are quite comparable in the two pathways. The chemical simulation of step III is quite significant in that it demonstrates successfully the use of aspartate as an amino group donor, a phenomenon that is very rare in Nature (ref.3). Only three such cases are known and two of these form part of the ATP-Imidazole cycle.

Remarkable similarity can be seen in the enzymatic and simulation pathways (Figure I), in terms of activation, aspartate addition and amino transfer. A careful analysis of adenylysuccinate mediated amino transfer with concomitant formation of fumarate has shown that the process is a concerted one and very close to an E_2 elimination. In the chemical simulation this aspect has been successfully exploited in the use of butyric acid which acts as a weak acid, generating an effective conjugate base. The superiority of the enzyme mediated process is evident in step IV involving specific N-1 alkylation with ribose. This process with the appropriate equivalent bromoacetone led to N-1 as well as 6-amino alkylation. The latter pathway however did not interfere with the overall cyclic operation. The enzymatic and simulation pathways diverge after the alkylation step. The protocol in the enzyme mediated pathway is, hydrolysis of the purine 1-6 bond (step V), isomerization (step VI), glutamine mediated amino transfer (step VII) and further cyclization and separation from the parent leading to the daughter imidazole product (step VIII). In the chemical simulation, the alkylated product with benzylamine and p-TsOH proceeds through equivalent stages giving rise to the daughter product in good yields. Evidence thus far seems to show that here the 1-6 bond cleavage occurs at a later stage.

Extended, painstaking and highly perceptive investigations carried out by Sarah Ratner and her colleagues have provided a wealth of information pertaining to each of the operations involved in the urea cycle. A comparison of the enzymatic (ref.4) and chemical simulation (ref.5) (Figure II) would show remarkable similarities. The chemical methodology for amide transfer, which initiates the urea cycle, leading to the transformation of ornithine to citrulline, closely parallels the *in vivo* operations mediated by transcarnylase. The latter makes use of carbamoyl phosphate as the reagent which would bring about the desired change by loss of phosphate, an excellent leaving group. This is reflected in the high K_{eq} (100,000) for the reaction. The choice of nitrourea for amide transfer in the chemical simulation takes advantage of the irreversible nature of the process, in the sense that the departing entity is rapidly transformed to N_2O and H_2O . Both processes are effective under ambient pH conditions and the yield in the chemical simulation is satisfactory.

Argininosuccinate synthetase which mediates the conversion of citrulline to argininosuccinate is a remarkable enzyme composed of two pairs of cross linked monomers. In its ability to accept sequentially ATP, citrulline and aspartate, the profile closely resembles that of aminoacyl synthetases involved in linking of t-RNA to coded amino acids. The K_{eq} (90) for this change is modest.

In view of the complexity of the process, the chemical simulation involving tosyl chloride mediated activation giving rise to δ -cyano ornithine, followed by aspartate addition to the product argininosuccinate, in overall 52% yield is noteworthy. An advantage in the chemical simulation is the isolation of the activated species δ -cyano ornithine. The transformation of this to argininosuccinate is



critically dependent on the pH of the medium. The low pH for optimal reaction suggests the protonated form of the substrate for aspartate addition. The possibility of δ -cyanoornithine or very closely related species in *in vivo* reaction can not be ruled out. This aspect is under examination. For a biological process the transformation of argininosuccinate to arginine is highly inefficient with a K_{eq} of 0.0114. The enzyme which mediates this, namely, argininosuccinase consists of 4 units and the optimum turnover is secured at biological pH. Against this background the corresponding change at pH 2.5 in the

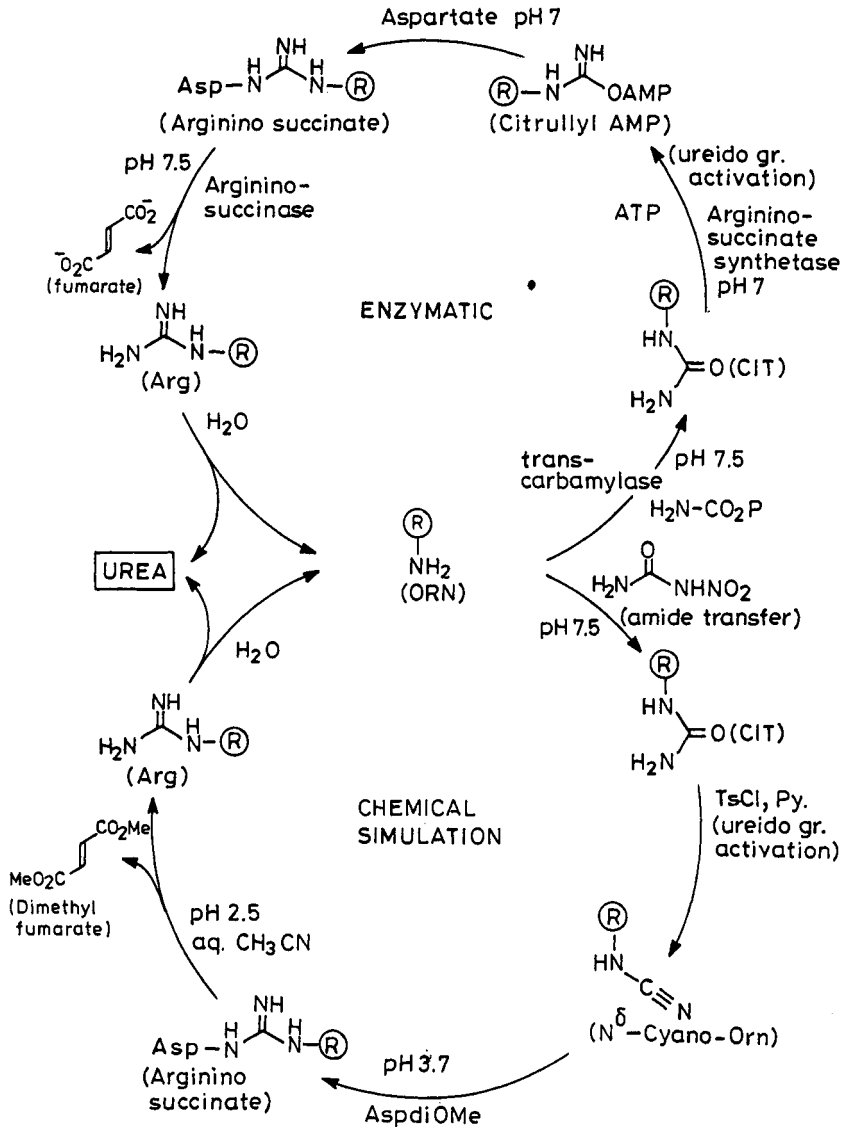


FIGURE II UREA CYCLE

chemical simulation experiment leading to 56% of the product is remarkable. The low pH for the reaction is not unexpected since a protonated guanidinium intermediate would be more susceptible for the required C-N bond scission of the aspartate moiety.

Central to the strategy in the chemical simulation of the α -amidating action of pituitary enzymes is the recognition that an appropriately placed C-C bond is likely to undergo scission compared to that of the C-H bond rupture in the *in vivo* operation (Figure III). Although not completely established, it appears plausible that the biological operation involves a primary, enzyme mediated $-\text{CO}-\text{NH}-\text{CH}_2-\text{COOR} \rightarrow -\text{CO}-\text{N}=\text{CH}-\text{COOR}$ change followed by non-enzymatic addition of water to the carbinol and cleavage. Thus, the enzyme PAM brings about the oxidation of a rather re-calcitrant substrate using strongly oxidizing Cu species generated from Cu(II), O₂ and ascorbic acid (ref.6). In the laboratory parallel, the $-\text{CO}-\text{NH}-\text{CH}(\text{CH}_2\text{OH})-\text{COOR} \rightarrow -\text{CO}-\text{N}=\text{CH}-\text{COOR}$ change was

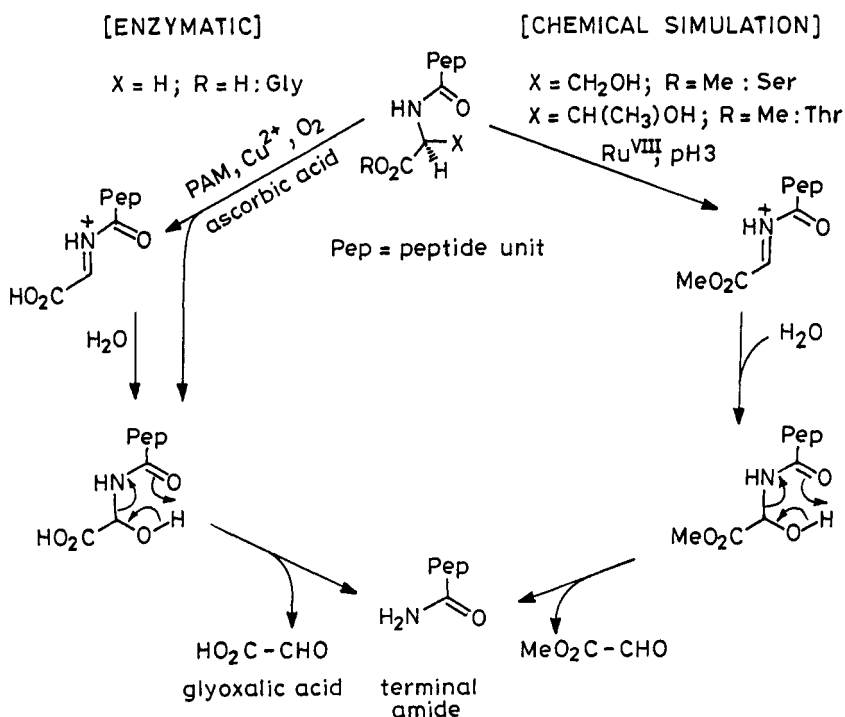


FIGURE III α -AMIDATING ACTION OF PITUITARY ENZYMES: GENERATION OF C-TERMINAL AMIDES FROM PROTEIN PRECURSORS

accomplished (ref.7) under relatively mild conditions with an overall yield of 70% of the product amide. The differences in the optimum pH pertaining to the two pathways can easily be attributed to the needs of the reagent.

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