# Stereospecificity of Thymidylate Synthetase

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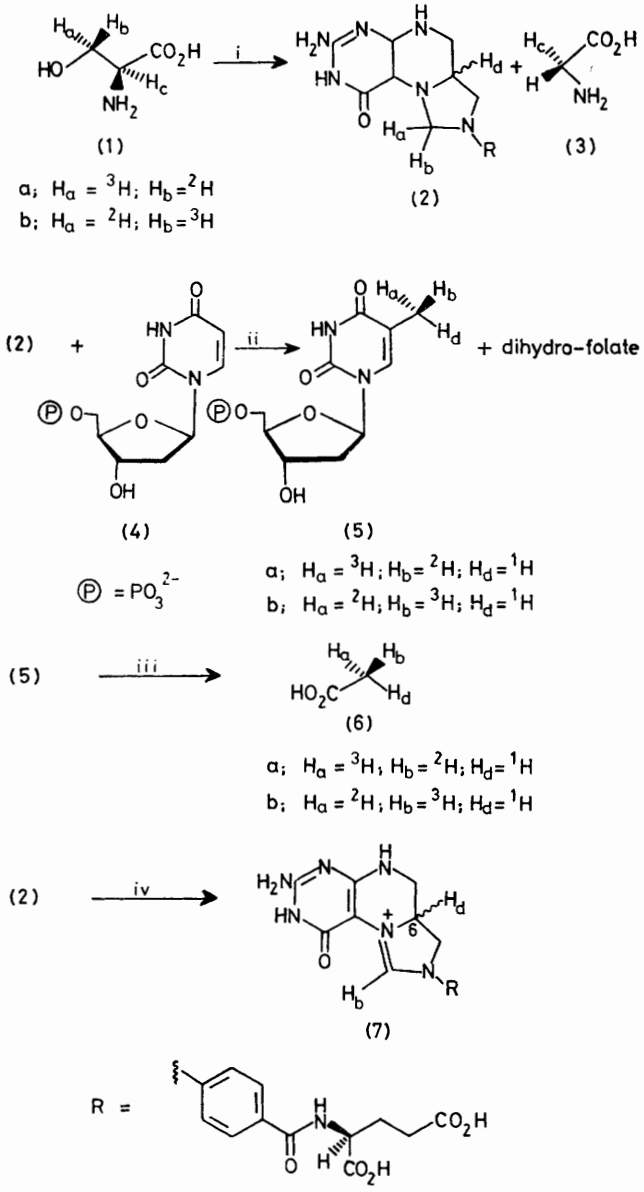
*Summary* The stereospecific formation of the methyl group of thymidylate from uridylate and 5,10-methylene-tetrahydrofolate catalysed by thymidylate synthetase has been delineated.

OUR interest in processes utilizing tetrahydrofolate led us to examine the stereochemistry of thymidine biosynthesis which is essential for DNA production. Earlier studies had shown that serine transhydroxymethylase (E.C.2.1.2.1)

transfers the prochiral β-methylene group of serine (**1**) to tetrahydrofolate to form 5,10-methylenetetrahydrofolate (**2**) with a stereospecificity of at least 75%.[1] In the next step thymidylate synthetase (E.C.2.1.1.b) adds this methylene group and the 6-hydrogen of (**2**) ($H_d$) to deoxyuridine monophosphate (**4**) to generate thymidine monophosphate (**5**) and dihydrofolate.[2] Knowledge of the final steric relationship of the heterotopic serine hydrogens ($H_a$ and $H_b$) and $H_d$ in the methyl group of thymidine would




i, Serine transhydroxymethylase; ii, thymidylate synthetase; iii, $KMnO_4$, $NaIO_4$; iv, dehydrogenase.

determine whether the thymidylate synthetase reaction is stereospecific and would give valuable information about possible configurational arrangements on the enzyme surface.

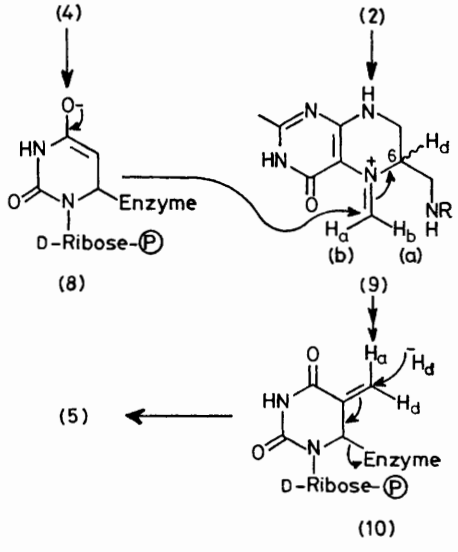
Our basic approach was to label each of the hydrogens $H_a$, $H_b$, and $H_d$ with a different isotope and analyse the chirality of the resulting methyl group. Thus (2*S*,3*R*)-[3-$^2$H,$^3$H]serine (**1a**) and (2*S*,3*S*)-[3-$^2$H,$^3$H]serine (**1b**) were synthesized using a modification of an earlier method for the preparation of stereospecifically tritiated serine,[3] in which deuterium is introduced *via* the hexose monophosphate isomerase reactions. The serine samples were shown to have high stereochemical purity by conversion into acetate using a known stereospecific sequence (serine → tryptophan → lactate → acetate)[4] and chirality analysis of the acetate using Eggerer's procedure.[5] The serines (**1a**) and (**1b**) were individually transformed by rabbit liver serine transhydroxymethylase and *L. casei* thymidylate synthetase in coupled reactions to thymidine monophosphate (**5**). Oxidation of these nucleotides after dilution with [$^{14}CH_3$]-thymidine monophosphate proceeded smoothly under Lemieux–von Rudloff conditions[6] to produce the chiral acetic acid (**6**) (15—30% yield), which was isolated by steam distillation. Chirality analysis of (**6**) showed that (3*R*)-serine (**1a**) had generated thymidine monophosphate (**5a**) possessing the *R* configuration in its methyl group; likewise the (3*S*)-isomer (**1b**) gave (**5b**).

TABLE. Stereochemical analyses: $^3H/^{14}C$ of degradation products

| | Serine | | Thymidine-P | | Control (acetate)[a] | |
|---|---|---|---|---|---|---|
| | (**1a**) | (**1b**) | (**5a**) | (**5b**) | (*R*) | (*S*) |
| Substrate | | | 3·10 | 2·35 | | |
| Acetate | 7·66 | 6·74 | 3·14 | 2·45 | 7·60 | 8·15 |
| Malate | 6·41 | 5·76 | 3·09 | 2·33 | 6·12 | 6·68 |
| Fumarate | 2·07 | 4·03 | 2·10 | 0·73 | 4·42 | 2·25 |
| % $^3H$ retention of fumarase reaction | 32·3 | 69·9 | 68·7 | 31·4 | 72·2 | 33·7 |

[a] Authentic samples of chiral acetate obtained from Prof. H. Eggerer, Regensburgh.

These results clearly demonstrate that the thymidylate synthetase reaction is highly stereospecific. In the overall process the 'glycine part' and the hydroxy group of serine are replaced with retention by the 6-hydrogen of tetrahydrofolate and the deoxyuridine monophosphate unit, respectively. The pyridoxal phosphate-dependent serine transhydroxymethylase probably fragments serine (**1**) to



generate enzyme-bound formaldehyde and a glycine-derived anion which is stereospecifically protonated from the *si* face [*i.e.* retention of configuration of $H_c$ in (**3**)].[7] Some of the formaldehyde may undergo rotation prior to condensation with tetrahydrofolate to form (**2**) which is partially racemized at the methylene carbon.[1] Because the configuration at C-6 in (**2**) is unknown, it is not possible to determine the absolute location of $H_a$ and $H_b$ in this compound, but it is reasonable to assume that its stereospecific oxidation by 5,10-methylenetetrahydrofolate dehydrogenase to methylidynetetrahydrofolate (**7**) which retains the *pro-S* hydrogen ($H_b$) of serine proceeds from the least hindered side. There is evidence that thymidylate synthetase adds in Michael fashion to deoxyuridine monophosphate (**4**) to give the enolate (**8**) which attacks the iminium ion (**9**) formed from (**2**) by this enzyme.[8] This addition product may cleave to the *exo*-methylene derivative (**10**) which stereospecifically accepts the 6-hydrogen ($H_d$) to produce thymidine monophosphate (**5**). With the present results and some reasonable assumptions, knowledge of the absolution configuration of 5,10-methylenetetrahydrofolate (**2**) would predict the three-dimensional orientations of the reaction components on the surface of serine transhydroxymethylase and thymidylate synthetase.

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