The Aminoacylation of Transfer Ribonucleic Acid

RECOGNITION OF METHIONINE BY *ESCHERICHIA COLI* METHIONYL-TRANSFER RIBONUCLEIC ACID SYNTHETASE

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The mechanism of the recognition of methionine by *Escherichia coli* methionyl-tRNA synthetase was examined by a kinetic study of the recognition of methionine analogues in the ATP-PP_i exchange reaction and the tRNA-aminoacylation reaction. The results show that the recognition mechanism consists of three parts: (1) the recognition of the size, shape and chemical nature of the amino acid side chain at the methionine-binding stage of the reaction; (2) the recognition of the length of the side chain at the stage of aminoacyl-adenylate complex-formation; (3) the recognition of the sulphur atom in the side chain at the stage of methionyl-tRNA formation. It is proposed that the sulphur atom interacts with the enzyme to induce a conformational change. A model of the active site incorporating the mechanism of methionine recognition is presented.

Although aminoacyl-tRNA synthetases each recognize specifically only one naturally occurring amino acid, many have been found to recognize a wide variety of amino acid analogues (Loftfield, 1972). A study of the recognition of a range of amino acid analogues in the reactions of aminoacyl-tRNA synthesis can indicate some of the properties of the amino acid side chain that are important in the specific interaction between an amino acid and its specific aminoacyl-tRNA synthetase (Old & Jones, 1976) and thus lead to an understanding of the recognition mechanism.

The reaction catalysed by most of the aminoacyl-tRNA synthetases occurs in three stages. The first stage is the binding of the amino acid and ATP to the enzyme (Allende *et al.*, 1970), and this is followed by the formation of an enzyme-bound aminoacyl-adenylate complex (Allende & Allende, 1971), accompanied by the release of PP_i. The recognition of an amino acid analogue in these two stages can be investigated by using the ATP-PP_i exchange reaction. The recognition of an analogue at the binding stage is measured by its K_m value in the reaction, and the V_{max} is a measure of the ability of the analogue to form an aminoacyl-adenylate complex. Some analogues such as amino acid esters and D-amino acids have been found to bind to the synthetase, but are unable to form an aminoacyl-adenylate complex (Bruton & Hartley, 1968; Owens & Bell, 1970). Such analogues will competitively inhibit the ATP-PP_i

exchange reaction. The third stage of the aminoacylation reaction is the binding of tRNA to the enzyme-bound aminoacyl-adenylate complex and the subsequent transfer of the amino acid to the tRNA molecule. Ebel *et al.* (1973) have proposed that the recognition of tRNA at this stage is a two-step mechanism. The first step is the binding of the tRNA molecule and possibly depends on the recognition of the overall shape of the tRNA molecule by the enzyme. The second step involves the ability of the tRNA molecule to be aminoacylated and may depend on some specific property of the nucleotide sequence that is recognized at the active site of the enzyme. The recognition of an amino acid analogue in the third step of the aminoacylation reaction could not be measured directly by using the aminoacylation reaction, since radiolabelled analogues were not available. However, it was measured indirectly by determining the ability of the analogue to inhibit the extent of aminoacyl-tRNA formation in the overall reaction (Old & Jones, 1976).

To study the recognition of methionine in the first two stages of the aminoacylation reaction we have compared the kinetic parameters exhibited by methionine with those of ethionine, homocysteine, norleucine, norvaline, α -aminobutyric acid, S-methylcysteine and S-ethylcysteine in the ATP-PP_i exchange reaction by using pure methionyl-tRNA synthetase from *Escherichia coli*. These results have been compared with the inhibitory effects of these analogues on the extent of methionyl-tRNA formation. From the results of these studies we have determined some of the features of the methionine side

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chain which are involved in the recognition mechanism and have incorporated these findings into a suggested model for the recognition of methionine at the active site of *E. coli* methionyl-tRNA synthetase and formation of the methionine-tRNA bond.

Materials and Methods

Chemicals

Reagent-grade chemicals were used. *S*-Ethyl-L-cysteine was a gift from Dr. J. S. Morley, Imperial Chemical Industries Ltd., Pharmaceuticals Division, Alderley Park, Macclesfield, Cheshire, U.K. DL-Homocysteine was purchased from Koch-Light Laboratories, Colnbrook, Bucks., U.K. All other amino acid analogues and D-amino acids were purchased from Sigma (London) Chemical Co., London S.W.6, U.K. L-[methyl- ^{14}C]Methionine (54 mCi/mmol) and tetrasodium [^{32}P]pyrophosphate (21.8 mCi/mmol) were obtained from The Radiochemical Centre, Amersham, Bucks., U.K. Scintillation fluid was composed of 5 g of 2,5-diphenyloxazole and 0.3 g of 1,4-bis-(5-phenyloxazol-2-yl)benzene (Packard Instruments, Caversham, Berks., U.K.) in 1 litre of analytical-grade toluene.

tRNA and aminoacyl-tRNA synthetase

Unfractionated tRNA prepared from *E. coli* by the method of Zubay (1962) was a gift from Dr. S. Nishimura, National Cancer Research Institute, Tokyo, Japan. Purified tRNA_f^{Met} and tRNA_m^{Met} were prepared by (1) chromatography of crude *E. coli* tRNA on DEAE-Sephadex A-50 (Pharmacia Fine Chemicals A.B., Uppsala, Sweden) by the method of Nishimura *et al.* (1967), which yielded highly purified tRNA^{Met}, and (2) chromatography of purified tRNA^{Met} obtained in step (1) on arginine-agarose (Jay & Jones, 1974), which separated the two tRNA^{Met} species, tRNA_f^{Met} and tRNA_m^{Met}. *E. coli* methionyl-tRNA synthetase was a gift from Dr. C. Bruton, Department of Biochemistry, Imperial College, University of London.

ATP-PP_i exchange assay

The ATP-PP_i exchange assay was carried out by the procedure described by Calendar & Berg (1966). The standard reaction mixture consisted of 0.1 M-Tris/HCl, pH 7.8, 2 mM-ATP, 5 mM-MgCl₂, 2 mM-KF, 10 mM-mercaptoethanol, 2 mM-[^{32}P]PP_i (10⁵ c.p.m./μmol) and contained 0.1 mg of bovine serum albumin and either L-methionine or an amino acid analogue as indicated, in a total volume of 1 ml. The reaction was initiated by adding 1.9 μg of pure methionyl-tRNA synthetase and the reaction mixture incubated at 37°C. The reaction was stopped after an appropriate time by the addition of 0.5 ml of 7% (v/v) HClO₄, and 0.2 ml of an acid-washed charcoal

suspension (15% dry weight in water) was added to the reaction mixture. After mixing thoroughly, the mixture was filtered through a Whatman GF/C glass-fibre disc. The disc was washed with 3 × 10 ml of water, dried on an aluminium planchet at 60°C for 15 min and assayed for radioactivity in a gas-flow counter (Nuclear-Chicago) equipped with a Micromil end window.

Assay of amino acid-acceptor activity of tRNA

The aminoacylation of tRNA and the effect of analogues on the extent of aminoacylation were assayed as described previously (Old & Jones, 1976), except that the synthetase preparation from *E. coli* was replaced by 1.5 μg of pure methionyl-tRNA synthetase and the crude tRNA was replaced by 0.05 A₂₆₀ unit of either purified tRNA_f^{Met} or tRNA_m^{Met}.

Results

Each analogue of methionine was first tested for its ability to support the ATP-PP_i exchange reaction. The results of these experiments are presented in Table 1. The amount of ATP-PP_i exchange stimulated by each analogue has been converted for comparison purposes into a percentage of the amount stimulated by methionine at one-twentieth the

Table 1. Stimulation of ATP-PP_i exchange by L-methionine and methionine analogues with pure *E. coli* methionyl-tRNA synthetase

Details of the assay procedure are presented in the Materials and Methods section. Standard reaction mixtures contained either 1 μmol of methionine or 20 μmol of an amino acid analogue. The amount of ATP-PP_i exchange is expressed in μmol of PP_i exchanged/15 min per mg of protein (the protein concentration was determined by Dr. Bruton, who gave us the enzyme) and is also expressed as a percentage of that stimulated by L-methionine under the conditions described.

Amino acid or analogue	Amount of exchange	Exchange (%)
None	2.9	0
L-Methionine	111.5	100
L-α-Aminobutyric acid	64.8	58
L-Ethionine	112.0	100
L-Norvaline	66.0	60
L-Norleucine	111.2	100
L-S-Methylcysteine	2.9	0
L-S-Ethylcysteine	6.4	3
DL-Homocysteine	116.1	105
D-Ethionine	5.3	2
D-Norvaline	3.0	0
D-Norleucine	3.6	1
D-Methionine	6.4	3
D-Leucine	2.1	0
D-Valine	4.5	2

concentration of each analogue. L-Ethionine, DL-homocysteine and L-norleucine each supported ATP-PP_i exchange by a similar amount, which was approximately equal to that stimulated by methionine at the lower concentration. Similarly, L-norvaline and L- α -aminobutyric acid supported the reaction to the lesser extents of 58 and 60% respectively. L-S-Methylcysteine and L-S-ethylcysteine were found to be inactive in the reaction. Table 1 also shows the results of a number of D-amino acids which were tested for their suitability to support ATP-PP_i exchange with pure methionyl-tRNA synthetase by the same method. Allowing for experimental error, D-methionine, D-norvaline, D-norleucine and D-ethionine were found not to support any ATP-PP_i exchange.

The kinetic parameters for L-methionine and the methionine analogues which were found to support ATP-PP_i exchange in Table 1 were determined with pure methionyl-tRNA synthetase by the method of Lineweaver & Burk (1934). For each analogue the rates of ATP-PP_i exchange were linear for the initial 10 min. Reciprocal plots of the exchange rate and the substrate concentration were drawn for each analogue and the Michaelis constants calculated. The K_m and V_{max} values for each analogue are presented in Table 2. The K_m value of 0.02 mM obtained for methionine compares favourably with the previously reported values of 0.04 mM (Lemoine *et al.*, 1968) and 0.07 mM (Bruton & Hartley, 1968). Ethionine and

norleucine had K_m values of 4 and 7 mM respectively, agreeing with the K_m values reported by Lemoine *et al.* (1968) of 5 and 8 mM respectively. Homocysteine had a K_m value of 13 mM, whereas norvaline and α -aminobutyric acid both had a higher K_m value, of 100 mM. All five analogues had the same V_{max} value, which was significantly lower than the V_{max} of methionine under the same conditions.

The methionine analogues that did not support ATP-PP_i exchange, L-S-methylcysteine, L-S-ethylcysteine, D-methionine, D-ethionine, D-norleucine and D-norvaline, were tested for their ability to inhibit methionine-stimulated ATP-PP_i exchange by the method of Lineweaver & Burk (1934). No inhibition was observed with any of these analogues. The results for the four D-amino acids confirm that methionyl-tRNA synthetase exhibits a strict stereospecificity for the L-isomers of methionine and methionine analogues at the methionine-binding site.

The effect of each methionine analogue on the extent of methionyl-tRNA formation by using an *E. coli* extract as the synthetase preparation and crude *E. coli* tRNA has been reported (Old & Jones, 1976). Similar results have been obtained by using pure methionyl-tRNA synthetase and purified tRNA^{Met} and tRNA^{Met} species from *E. coli*. Table 3 compares the inhibition of formation of methionyl-tRNA^{Met} and methionyl-tRNA^{Met} by these analogues at an analogue/methionine molar ratio of 1000:1. Their efficiency of inhibition is in the order: ethionine (approx. 85%), homocysteine (approx. 60%), norleucine (approx. 20%) and norvaline and α -aminobutyric acid (approx. 6%).

Ethionine and norleucine have been shown pre-

Table 2. Kinetic parameters of the stimulation and inhibition of ATP-PP_i exchange by methionine analogues

For the determination of K_m values, standard reaction mixtures contained either methionine at concentrations ranging from 1.0 to 0.01 mM or an analogue at concentrations ranging from 10 to 0.1 mM. For the determination of K_i values, standard reaction mixtures contained 4 μ mol of the methionine analogue together with L-methionine at a range of concentrations varying from 1 to 0.01 mM. Michaelis constants and maximum velocities were determined by the reciprocal-plot method of Lineweaver & Burk (1934). Maximum velocities are expressed as μ mol of PP_i exchanged/10 min per mg of protein. L-S-Methylcysteine, L-S-ethylcysteine, D-methionine, D-ethionine, D-norleucine and D-norvaline did not inhibit ATP-PP_i exchange.

Analogue or amino acid	Stimulation	
	K_m (mM)	V_{max}
L-Methionine	0.02	90
L-Ethionine	4	60
L-Norleucine	7	60
DL-Homocysteine	13	60
L- α -Aminobutyric acid	100	60
L-Norvaline	100	60

Table 3. Effect of analogues on the formation of methionyl-tRNA species

Results are expressed as percentage inhibition of methionyl-tRNA formation in the presence of an analogue/methionine molar ratio of 1000:1. For tRNA^{Met} and tRNA^{Met}, assays were performed as described in the Materials and Methods section. Results for unfractionated tRNA are those reported previously (Old & Jones, 1976) for a crude synthetase preparation.

Analogue	Inhibition (%)		
	tRNA ^{Met}	tRNA ^{Met}	Unfractionated tRNA
L-Ethionine	89	85	84
L-Homocysteine	64*	58*	58*
L-Norleucine	21	17	20
L-Norvaline	7	6	6
L- α -Amino-butyric acid	6	8	6

* These values included a correction to allow for the fact that the DL-racemate was used in the assays (see Old & Jones, 1976).

viously to be esterified to tRNA in place of methionine (Trupin *et al.*, 1966). To determine whether analogues which inhibited the extent of methionyl-tRNA formation (Table 3) are also esterified to tRNA in place of methionine, each analogue was examined by the following criteria. To inhibit the extent of aminoacylation of tRNA, the inhibitor must bind either reversibly or irreversibly to the enzyme. If it binds reversibly but is not esterified to the tRNA molecule, it will inhibit the initial rate of aminoacylation, but not the extent. This possibility is ruled out in our case, as each of the methionine analogues in question has been shown to inhibit the extent of methionyl-tRNA formation (Old & Jones, 1976). If the inhibitor binds reversibly and is then transferred to the tRNA molecule, as with ethionine and norleucine, it will inhibit both the initial rate and the extent of aminoacylation. However, if the inhibitor binds irreversibly to the amino acid-binding site on the enzyme, the rate and extent of aminoacylation will also be decreased. A 'double-incubation' assay was developed as a simple means of distinguishing between the latter two types of inhibition and is based on the following reasoning. If the inhibitor functions by remaining bound to the enzyme, after 25 min incubation the maximum extent of aminoacylation has been reached, with some unesterified tRNA^{Met} remaining. Therefore the addition of extra tRNA will not increase the amount of [¹⁴C]methionyl-tRNA in the incubation mixture, but the addition of enzyme should. If the inhibitor functions by its esterification to tRNA^{Met}, the addition of extra tRNA will increase the amount of [¹⁴C]methionyl-tRNA, but the addition of extra enzyme will not.

Table 4 shows the results of the 'double-incubation' assay for ethionine, norleucine, homocysteine, norvaline and α -aminobutyric acid. With ethionine, the presence of the analogue decreased the extent of methionyl-tRNA formation after 25 min incubation, from an average value of 29.2 to 20.9 pmol. The addition of enzyme and further incubation for 25 min resulted in no further increase in the amount of methionyl-tRNA. However, the addition of an equal amount of tRNA and further incubation for 25 min increased the amount of methionyl-tRNA formed to 42.1 pmol, exactly double the amount formed in the first incubation. A similar result was obtained for norleucine, homocysteine, norvaline and α -aminobutyric acid. With each analogue, the addition of tRNA in the second incubation increased the amount of methionyl-tRNA formed to exactly twice that of the lower amount formed in the first incubation, whereas the addition of enzyme in the second incubation resulted in no increase. Thus these results show that each of the analogues listed in Table 4 inhibits the formation of methionyl-tRNA by binding reversibly to the enzyme and being esterified to tRNA^{Met} in the place of methionine.

Table 4. *Effect of methionine analogues on the extent of methionyl-tRNA formation in a two-step incubation assay*

For each methionine analogue indicated, two basic reaction mixtures were made up, each containing the analogue at a concentration that had been found to inhibit the extent of methionyl-tRNA formation by approx. 30%. After 25 min incubation at 37°C, a 50 μ l sample from each reaction mixture was assayed for [¹⁴C]methionyl-tRNA as previously described (Old & Jones, 1976). For each analogue 0.15 mg of unfractionated tRNA was added to one reaction mixture and 0.06 mg of enzyme added to the other. Both reaction mixtures were reincubated for 25 min at 37°C and the extent of [¹⁴C]methionyl-tRNA formation assayed as above. The extents of methionyl-tRNA formation are expressed as pmol of methionine incorporated/ A_{260} unit of tRNA.

Analogue	Extent of methionyl-tRNA formation		
	First incubation	Second incubation	
		+tRNA	+Enzyme
None	29.6	—	29.0
None	28.8	58.1	—
L-Ethionine	21.0	—	21.1
L-Ethionine	20.8	42.1	—
DL-Homocysteine	18.2	—	18.8
DL-Homocysteine	19.0	42.6	—
L-Norleucine	23.8	—	25.4
L-Norleucine	21.6	42.4	—
L-Norvaline	17.7	—	18.6
L-Norvaline	17.7	36.9	—
L- α -Aminobutyric acid	18.0	—	19.4
L- α -Aminobutyric acid	18.4	34.0	—

Discussion

Our previous work (Old & Jones, 1976) with unfractionated tRNA and a crude synthetase preparation from *E. coli* examined the effect of analogues with different side-chain structures on the formation of methionyl-tRNA. From the results it was possible to make some proposals as to the features in the amino acid side chain that are recognized by the synthetase. Here we show that similar results are obtained with pure methionyl-tRNA synthetase and that the effect of the analogues on the esterification of either tRNA_f^{Met} or tRNA_m^{Met} is the same. This latter result is not surprising, since it is the same enzyme which esterifies both species of tRNA.

The availability of pure methionyl-tRNA synthetase (kindly given by Dr. C. Bruton) has enabled us to examine the behaviour of these analogues not only in the overall aminoacylation reaction but also in the first two stages of the reaction sequence, namely the binding of methionine to the synthetase and the formation of aminoacyl-AMP-synthetase

complex. From this it has been possible to distinguish features of the side chain which appear to be important at each stage of the overall reaction.

As would be predicted, the five methionine analogues that inhibit the formation of methionyl-tRNA stimulate the ATP-PP_i exchange reaction. To measure the recognition of each analogue at the methionine-binding site, the K_m value for each analogue was determined. Ethionine, norleucine and homocysteine had similar K_m values, showing that the enzyme recognizes each of the three analogues to a similar extent. However, the K_m value for methionine was at least two orders of magnitude lower, showing that the enzyme has a much greater binding affinity for methionine than for ethionine, norleucine and homocysteine. The side chain of norleucine is of a similar size and shape to that of methionine, but it does not possess a sulphur atom. Ethionine and homocysteine have side chains that are different in size to that of methionine, but each possesses a sulphur atom in the same position of the side chain as the sulphur atom in methionine. Norvaline and α -aminobutyric acid have the same K_m values, which are an order of magnitude higher than those of L-ethionine, L-norleucine and DL-homocysteine. The side chains of these analogues are shorter than methionine and lack a sulphur atom.

S-Methylcysteine and S-ethylcysteine were found neither to stimulate ATP-PP_i exchange (Table 1) nor to inhibit methionine-dependent ATP-PP_i exchange. This shows that they cannot bind to the methionine-binding site and thus they are not recognized by the enzyme in the first stage of aminoacylation. S-Methylcysteine and S-ethylcysteine possess a sulphur atom in their side chains in a position one place nearer to the amino acid backbone than that in methionine.

These results suggest that, in the formation of the aminoacyl-AMP-synthetase complex, some variability in the length of side chain is permissible. The sulphur atom may be replaced by carbon (norleucine), oxygen (methoxinine; Richmond, 1962) or selenium (selenomethionine; McConnell & Hoffman, 1972); however, when present in the side chain, its position is critical.

The maximum velocity exhibited by an analogue in the ATP-PP_i exchange reaction is a measure of its ability to form an aminoacyl-adenylate complex. The methionine analogues had the same V_{max} value, which was only two-thirds the V_{max} of methionine under the same conditions. Thus there is a recognition mechanism at the second stage of aminoacylation which discriminates equally against the five methionine analogues. As the side chain of each analogue, even norleucine, is not identical in length with the methionine side chain, one possible explanation of this recognition mechanism is that when each analogue is bound at the methionine-binding site, the

carboxyl group is not in the optimum position to react with the bound ATP molecule. Thus each analogue will form an aminoacyl-adenylate complex at a slower rate than methionine. By this reasoning an analogue with a side chain identical in length with that of methionine will exhibit the same V_{max} as methionine. One such analogue is selenomethionine, which has a side chain very similar in length to that of methionine. Here selenium replaces sulphur and these atoms have a more similar covalent radius than have sulphur and carbon (Pauling, 1947), the latter being the atom which replaces sulphur in norleucine. Bruton & Hartley (1968) have shown that selenomethionine and methionine exhibited the same V_{max} value in the ATP-PP_i exchange reaction, and that the V_{max} exhibited by norleucine was one-half of this value.

The effect of an analogue on the extent of methionyl-tRNA formation is a measure of the recognition of the analogue in the overall aminoacylation reaction. Thus the efficiency of an analogue in effecting ATP-PP_i exchange should be reflected in its ability to inhibit methionyl-tRNA formation.

The ratio of the K_m value for each analogue to the K_m value for methionine in the ATP-PP_i exchange reaction and the analogue/methionine molar ratios required to give 50% inhibition of methionyl-tRNA formation show a close relationship, except for norleucine. Ethionine and homocysteine give K_m ratios of 200:1 and 650:1 respectively, and require molar ratios of 220:1 and 800:1 respectively (analogue/methionine) to give 50% inhibition of methionyl-tRNA formation in the aminoacylation reaction. Norvaline and α -aminobutyric acid give much larger K_m ratios (5000:1 in each case) and require an analogue/methionine molar ratio of 10000:1 to give just less than 50% inhibition of methionyl-tRNA formation. S-Methylcysteine and S-ethylcysteine neither support ATP-PP_i exchange nor inhibit methionyl-tRNA formation. Norleucine, however, has a K_m ratio of 350:1, whereas to give 50% inhibition of methionyl-tRNA formation, an analogue/methionine ratio of 8000:1 is required.

Therefore except for norleucine it appears that the major difference in recognition of each analogue by the synthetase is in the first step of the reaction. (Possibly the difference in the values of the two ratios is in part due to the slight difference in V_{max} values of methionine and the analogues.)

For norleucine, another recognition factor appears to be involved after the formation of the aminoacyl-adenylate complex. This factor permits the enzyme to distinguish norleucine from ethionine and homocysteine and decreases the recognition of norleucine to the extent of recognition of norvaline and α -aminobutyric acid. Thus in the recognition of methionine by *E. coli* methionyl-tRNA synthetase, there is a

Fig. 1. Schematic representation of the active site of *E. coli* methionyl-tRNA synthetase. For an explanation, see the text.

recognition mechanism at the third stage of aminoacylation that allows the enzyme to distinguish between methionine and norleucine but not between methionine and ethionine or homocysteine. Our results indicate that it is the sulphur atom that is the recognition factor. Ethionine and homocysteine each possess a sulphur atom in the same position as methionine, whereas norleucine lacks a sulphur atom. *E. coli* methionyl-tRNA synthetase has been shown to consist of two identical subunits (Koch & Bruton, 1974) and thus the enzyme may be subject to allosteric control mechanisms. The recognition mechanism at the third stage of aminoacylation can be explained by such a mechanism.

Fig. 1 shows a suggested schematic representation of the active site of methionyl-tRNA synthetase incorporating the mechanisms of methionine recognition. It is proposed that the methionine-binding site is a rigid pocket just large enough to accommodate the methionine side chain. Fig. 1(a) shows methionine bound to the active site with its carboxyl group optimally placed for reaction with the bound ATP molecule. The methionine-binding site contains an amino acid (labelled R in Fig. 1, as our results give no indication as to the nature of the amino acid) which is positioned adjacent to the sulphur atom of the bound methionine side chain. The sulphur atom interacts with the side chain of amino acid R and the interaction induces a conformational change in the enzyme, represented in Fig. 1(b). The conformational change is illustrated by a pivoting at a hinge point between the methionine-binding site and the tRNA-binding site, resulting in the movement of the bound tRNA molecule to the optimum position for its reaction with the methionyl-adenylate complex. Norleucine lacks a sulphur atom and thus cannot induce this conformational change in the enzyme, resulting in a decrease in the ability of the norleucyl-

adenylate complex to react with the tRNA molecule. As the formation of the aminoacyl-adenylate complex is an equilibrium reaction, with the reverse reaction being much faster than the forward reaction (Santi *et al.*, 1974), a decrease in the ability of the norleucyl-adenylate complex to react with tRNA will result in a greater breakdown rate of the norleucyl-adenylate complex, thus decreasing the amount of norleucine esterified to tRNA in comparison with ethionine and homocysteine.

The recognition of each methionine analogue can be explained by the model in Fig. 1. Ethionine has a sulphur atom in the same position as methionine, but the side chain is longer. Thus when ethionine is bound at the methionine-binding site, the sulphur atom is positioned slightly above the amino acid R, but is close enough to interact with it. Homocysteine can also bind with its sulphur atom in the correct position to induce a conformational change. Norleucine, norvaline and α -aminobutyric acid can all bind to the methionine-binding site, but they cannot induce the conformational change. *S*-Methylcysteine and *S*-ethylcysteine will not fit into the methionine-binding site of the proposed model. If either analogue is positioned in the methionine-binding site with the sulphur atom adjacent to the amino acid R, the amino group assumes a horizontal position and is sterically hindered by the wall of the methionine-binding site.

Thus, to summarize, our results indicate that the mechanism of methionine recognition by *E. coli* methionyl-tRNA synthetase consists of three steps. The first is at the binding of methionine to the enzyme and involves the recognition of the size, shape and chemical nature of the methionine side chain. The second step is at the formation of the aminoacyl-adenylate complex and involves the correct positioning of the carboxyl group for the reaction to take place. The third is at the stage of methionyl-

tRNA formation and involves the recognition of the sulphur atom in the methionine side chain. It is proposed that the sulphur atom interacts with a specific amino acid at the methionine-binding site, inducing a conformational change in the enzyme, resulting in the movement of the bound tRNA molecule to the optimum position for the reaction to take place. A conformational change is the most likely explanation of our results, although other explanations are possible. For example, the sulphur atom could be recognized by the tRNA molecule instead of by the enzyme. If such an interaction is shown to exist, the proposed model is such that it can be easily adapted in the light of further results.

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