The liver microsomal cholesterol 7α-hydroxylase has been found to have a K_m for O_2 about 20 μM and a K_m for NADPH about 300 μM. The activation energy of this hydroxylase has been shown to be 22±4kcal/mol, which is of the same order as those of certain other sterol hydroxylases determined in this laboratory (J. I. Mason & G. S. Boyd, unpublished work). The cholesterol 7α-hydroxylase system requires the presence of certain thiols, such as β-mercaptoethylamine, for optimum activity. In the absence of these specific thiols an 'autoxidative attack' occurs on the substrate, somewhat similar to a photosensitized reaction. This moderating effect of β-mercaptoethylamine, which is known to be efficient in scavenging radicals as a radiobiological protecting agent, suggests that thiols or thiyls may play an important part in the generation and steering of the reaction complex.

Until we have a highly purified sample of cytochrome *P*-450 and possibly also developments in physical methods for the investigations of these complex structures our concepts of the nature of the oxygen-attacking species in hydroxylation reactions will be largely conjecture. The oxygen molecule has to accept two electrons in the 'activation' process, and as this process is likely to occur stepwise it seems that O_2^- will be an intermediate (cf. Bray, 1970). It has been suggested that biological hydroxylation reactions have much in common with oxene (·Ö·) hydroxylations (Ullrick & Staudinger, 1966, 1969). On the above evidence it is at least possible to speculate that the second electron could be donated to the oxygen via a thiol, either as part of the cytochrome *P*-450 molecule or from an external source. The absence of certain specific thiols may be the reason for certain biological hydroxylation reactions yielding aberrant products (cf. Scholan & Boyd, 1968).

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COMMUNICATIONS

The Subunits of Methionyl-Transfer-Ribonucleic Acid Synthetase

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The methionyl-tRNA synthetase of *Escherichia coli* has a molecular weight of 180000 in its native form (Heinrikson & Hartley, 1967) and consists of four subunits (Lemoine, Waller & van Rapenbusch, 1968). Enzymically active monomers and dimers have been prepared (Cassio & Waller, 1968; Bruton & Hartley, 1968), but it has not been shown whether the subunits are identical, nor has the nature of the interconversion of the tetramer into dimer or monomer been established.

We have purified methionyl-tRNA synthetase in its dimeric form as described previously (Bruton & Hartley, 1968) and in the tetrameric form by omitting the autolytic stage in the purification procedure. Both preparations were homogeneous as judged by ultracentrifugation and polyacrylamide-gel disc electrophoresis. Sedimentation-equilibrium studies showed that the molecular weights of the native and maleylated tetramer were 179000 and 45000 respectively.

[^{14}C]Maleyl-protein was prepared from both the preparations and digested with thermolysin. The digests were acidified and applied to Dowex 50 columns equilibrated with 0.1M-acetic acid. The columns were eluted with 0.1M-acetic acid and the breakthrough tubes pooled. High-voltage paper ionophoresis at pH 6.5 revealed that these fractions contained three radioactive components, which for the dimeric form were identified as maleic acid, maleamic acid and a maleyl-peptide. From the tetramer three maleyl-peptides were found, but one

of these accounted for more than 95% of the radioactivity and had a mobility identical with that of the maleyl-peptide obtained from the dimer. Less than 5nmol of these peptides was available, but a micro 'dansyl'–Edman technique for determining the sequence was developed by scaling down the standard technique (Gray, 1967) to permit the detection of 10pmol of DNS-amino acid. By using this procedure the peptides, after being unblocked, were shown to have the sequence Ala-Gly-Gly-Thr.

As only one *N*-terminal sequence was detected, it is highly probable that the subunits of methionyl-tRNA synthetase are identical. Also, since the *N*-terminal sequences of both the dimer and the tetramer are the same, the conversion of tetramer into dimer cannot involve proteolytic cleavage except from the *C*-terminus.

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Heat Inactivation of Lysyl-Transfer-Ribonucleic Acid Synthetase and Arginyl-Transfer-Ribonucleic Acid Synthetase from Yeast: Evidence for Substrate-Induced Conformational Changes

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A 400-fold-purified preparation of lysyl-tRNA synthetase [L-lysine–tRNA ligase (AMP), EC 6.1.1.6] from baker's yeast (Chlumecká, von Tigerstrom, D'Obrenan & Smith, 1969) was protected against heat inactivation by L-lysine alone but not by normal substrate-level concentrations of ATP and $MgCl_2$ or tRNA and $MgCl_2$. The heat stability of the enzyme in the presence of lower concentrations of L-lysine was increased considerably by addition of ATP and $MgCl_2$ or tRNA and $MgCl_2$. Studies of the rate of heat inactivation of the enzyme in the presence of different concentrations of L-lysine suggested that the characteristics of binding of the amino acid to the enzyme in the absence of other substrates were different from those found in the presence of ATP and $MgCl_2$ or tRNA and $MgCl_2$. The experiments also suggested that the binding of tRNA to the enzyme in the presence of L-lysine was stronger than that found in the absence of the amino acid.

Experiments carried out with a partially purified preparation of arginyl-tRNA synthetase [L-arginine–tRNA ligase (AMP), EC 6.1.1.–] from baker's yeast (Mitra & Smith, 1969) showed different patterns of protection by substrates against heat inactivation from those found with the lysine enzyme. For the arginyl-tRNA synthetase maximum protection was observed in the presence of L-arginine, tRNA and $MgCl_2$ whereas L-arginine, ATP and $MgCl_2$ gave little protection. These results, suggesting that the binding of L-arginine is rather weak in the absence of tRNA, are consistent with other results (S. K. Mitra, A. H. Mehler & K. Chakraburtly, unpublished work) and with the observed absolute requirement for tRNA in the activation of arginine by arginyl-tRNA synthetase (Mitra & Mehler, 1967; Mitra & Smith, 1969).

The kinetics of inactivation of lysyl-tRNA synthetase and arginyl-tRNA synthetase may be interpreted in terms of conformational changes that are facilitated by the presence of tRNA and $MgCl_2$ or ATP and $MgCl_2$ and result in enhanced binding of amino acid.

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Possible Selective Genetic Controls in Synthesis of Messenger Ribonucleic Acid by Means of 'Paired' Histones along the Wide Groove of the Double Helix of Deoxyribonucleic Acid

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The original proposal of Stedman & Stedman (1950) that histones can act as genetic repressors has been extensively considered in terms requiring unmasking of the DNA genetic template by total removal of histones from their association with DNA (for extensive references see Busch, 1965; de Reuck & Knight, 1966; Bonner *et al.* 1968). I have proposed that unmasking of specific parts of the DNA template need not involve total separation of the histone from the DNA; instead a histone may be turned on its ionic linkage (of guanidinium or ammonium groups with the DNA phosphate group, in a manner similar to the opening of a door; Lewin, 1968, 1969).

As a result of construction of numerous correctly proportioned molecular models (Courtauld and