Myoglobin Variants

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The study of human haemoglobin variants has led to some insight into the variation of human genes. Some of the mutations are known to cause disease and others appear to be clinically benign. Some mutations are known to alter the physiological properties of the haemoglobin molecule. All known substitutions can be explained on the basis of single-point mutation in the DNA codon, but some of these are more frequent than others. Thus there are more changes involving the replacement of guanine by adenine in the mRNA than expected (Lehmann & Carrell, 1969).

It is therefore of interest to assess the frequency and type of other human protein variants. We have investigated nearly 6000 samples of human myoglobin by paper and acrylamide-gel electrophoresis. Three variants have been observed, but of necessity these methods will only detect substitutions producing a change of molecular charge. One would expect many neutral substitutions to remain undetected. Of the three variants it has been possible to identify two: 53 (D4) Glu $\rightarrow$ Lys (Boulton, Huntsman, Lorkin & Lehmann, 1969) and 138 (H16) Arg $\rightarrow$ Gln; both being guanine-to-adenine mutations of the mRNA.

During the survey a new haemoglobin was found in an English family, namely I-High Wycombe, with a mutation of residue $\beta 59$ (E3) Lys $\rightarrow$ Glu. This haemoglobin was easily identified because the tryptic hydrolysis between the tryptic peptides βV ($\beta 41-59$), which contains methionine, and $\beta TpV1$ ($\beta 60-61$) does not occur in the absence of the lysine residue at position $\beta 59$, and a new methionine-containing peptide $\beta 41-61$ was observed which was considerably more negatively charged than either peptide βTpV or peptide $\beta TpV1$ in normal haemoglobin. Amino acid analysis of this peptide showed the expected Lys $\rightarrow$ Glu replacement.

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The Mechanism of Action of Rat Liver Glutamyl-Transfer-Ribonucleic Acid Synthetase

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It has been suggested that the biosynthesis of aminoacyl-tRNA occurs in two stages, activation of the amino acids and their transfer to the tRNA (Hoagland, 1955; Hoagland, Zamecnik & Stephenson, 1957). The ATP-pyrophosphate exchange reaction has been considered to be a measure of the first stage. The participation of enzyme-bound aminoacyl adenylate as an obligatory intermediate (Wong & Moldave, 1960) was questioned by Loftfield & Eigner (1969) who studied the formation of aminoacyl hydroxamates. Salicylate has been shown to inhibit differentially the synthesis of aminoacyl-tRNA but to inhibit generally the ATP-pyrophosphate exchange reaction (Burleigh & Smith, 1970). Further, it has been shown that glutamate will not promote any ATP-pyrophosphate exchange in the absence of tRNA (Deutscher, 1967).

We have therefore studied the effects of salicylate on the glutamyl-tRNA synthetase activity present in a rat liver 105000g supernatant that has been passed through Sephadex G-25 to remove endogenous amino acids and ATP. The mechanism of inhibition was investigated by using fixed concentrations of two of the substrates and varying the third in the presence of different concentrations of salicylate. The drug showed a simple competitive inhibition with respect to glutamate and mixed competitive-non-competitive inhibitions with ATP and tRNA. These results are consistent with a mechanism for the action of the enzyme involving a compulsory addition of glutamate followed by the random addition of the other two substrates leading to the formation of a single quaternary complex (Fromm, 1967).

These observations strongly suggest that the esterification of glutamyl-tRNA involves the concerted interaction of the glutamate, ATP and tRNA, and similar considerations may apply to other aminoacyl-tRNA synthetases (Boyer, 1960). The formation of enzyme-bound aminoacyladenylate from certain amino acids, reflected by the ATP-pyrophosphate exchange, may occur as a side reaction.

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Comprehensible Models for Myoglobin and Haemoglobin

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The complexity of models of macromolecules built from Watson-Kendrew components is such that they are of limited value to those unused to viewing or building such models, particularly when viewing time is restricted. Conversely, Biobits molecular models are immediately comprehensible to both senior and junior workers and models of both myoglobin and haemoglobin will be shown.

Both models are suspended by practically invisible wire supports from frames such that the complete models are entirely visible; total weights and sizes of models are such that the complete units are readily picked up and orientated in any direction.

Myoglobin, based on the Kendrew co-ordinates, shows all helices and interhelical portions, the haem cleft or 'pocket', and the outer polar shell and every R group is immediately obvious. The haemoglobin model is built on two frames, each supporting an identical $\alpha\beta$ half-molecule so that the inner as well as the outer surfaces are readily available for study. Inter-subunit contacts are apparent, some being shown on the model whereas others are not. The myoglobin model may be built by two workers in about 2 days whereas the $\alpha\beta$ subunit requires about twice the time. Each individual R group or amino acid residue can be rapidly pinpointed and the lethal or benign effect of a mutational change can be readily understood or deduced.

The models, while being extremely stable, are flexible and mobile. Hence changes due to oxygenation, the Bohr effect etc. can be demonstrated by simply pushing parts of the model, which spring back on being released, in an allosteric manner. We have built a range of models of other proteins based on X-ray data, as well as of DNA and tRNA species etc.

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