Since lipoamide dehydrogenase fulfills the same service function in the pyruvate dehydrogenase and 2-oxoglutarate dehydrogenase multienzyme complexes, where it plays no part in the initial substrate specificity, the economy of a single structural gene makes good sense. This principle of parsimony (Perham, 1975) can be exemplified by the presence of a common subunit in structures where its molecular purpose and properties are still obscure and, in perhaps its ultimate form, in the existence of overlapping genes. In the papers that follow in this Colloquium, this theme will, I hope, unify what is at first sight an unwieldy range of topics for a single meeting.

We thank the Medical Research Council for Research Studentships to J. P. B. and R. A. H., and the Science Research Council for financial support.

Reed, L. J. & Oliver, B. M. (1968) Brookhaven Symp. Biol. 21, 397–412

Function of Thioredoxin in Oxidoreductions and as a Subunit of Phage-T7 Deoxyribonucleic Acid Polymerase

ARNE HOLMGREN and GUN-BRITT KARLSSON

Department of Chemistry, Karolinska Institute, S-104 01 Stockholm, Sweden

Thioredoxin is a small (mol. wt. 12000), ubiquitous electron-transport protein originally discovered in Escherichia coli as hydrogen donor for the enzyme ribonucleoside diphosphate reductase (Laurent et al., 1964). The functional group of thioredoxin consists of a single cystine residue that in the oxidized protein (thioredoxin-S2) forms a disulphide bridge of defined structure (Holmgren et al., 1975). The specific enzyme thioredoxin reductase catalyses the reduction of thioredoxin by NADPH, which gives the reduced form [thioredoxin-(SH)2] (Reichard, 1968). Thioredoxin-(SH)2 is a good biological reductant. Apart from its function in DNA synthesis as hydrogen donor for ribonucleotide reductase, it has also been shown to be involved in the enzymic reduction of methionine sulphone and sulphate in yeast (Gonzales Porqué et al., 1970). In addition to these enzymic reductions, thioredoxin-(SH)2 spontaneously reduces disulphide bonds in proteins like insulin (Holmgren & Morgan, 1976) and will thus be of importance...
for the overall thiol–disulphide status in the cell. More recently a regulatory function of thioredoxin in certain enzymic reactions has been suggested by the work of Wolosiuk & Buchanan (1977). They identified a chloroplast thioredoxin that acts in the light–ferredoxin–linked regulation of enzymes of photosynthetic CO₂ assimilation. Chloroplast fructose 1,6-bisphosphatase was activated by thioredoxin-(SH)₂ through reduction of a disulphide bond in the oxidized inactive enzyme.

The structure of thioredoxin-S₂ from *E. coli* has been determined by X-ray crystallography to a resolution of 0.28 nm (2.8 Å) (Holmgren et al., 1975). The molecule has 108 amino acid residues, most of which are located in the form of secondary-structure elements consisting of a central core of five strands of β-pleated sheet surrounded by four α-helices. The oxidation-reduction functional disulphide bond that is formed from cystine-32 and cystine-35 is located in a protrusion of the molecule. The C-terminal third of the molecule consists of two antiparallel strands of β-pleated sheet flanked by a terminal α-helix and appears as an independent folding unit of self-stabilizing capacity. In the crystalline state, thioredoxin forms dimers. The contact areas of the dimers are formed from homologous interactions of the C-terminal α-helix and are of a nature frequently observed in protomeric proteins. This potential site for protein–protein interaction in the thioredoxin structure was suggested to be utilized by thioredoxin for association with other enzyme subunits *in vivo* (Holmgren et al., 1975).

A novel function of *E. coli* thioredoxin in DNA synthesis was discovered through work on prototrophic mutants of *E. coli* that could not grow phage T7 (Chamberlin, 1974). One class of such mutants (tsnC) were blocked in the phage DNA replication owing to a defective host component necessary for a phage-induced DNA polymerase to be active (Modrich & Richardson, 1975). Purification of this host component was achieved by a complementation assay from wild-type cells. Nearly homogeneous preparations of the TsnC protein were shown by a number of criteria to be identical with *E. coli* thioredoxin (Mark & Richardson, 1976). The functional phage-T7 DNA polymerase was also isolated and shown to be a dimer containing 1 mol of the phage-T7-coded gene-5 protein (mol.wt. 84000) and 1 mol of *E. coli* thioredoxin. The gene-5 protein of the phage-T7 DNA polymerase showed no activity in either replication of phage-T7 DNA *in vitro* or a standard DNA polymerase assay with heat-denatured salmon spermatozoa DNA as template (Modrich & Richardson, 1975).

To study the function of the thioredoxin subunit of the phage-T7 DNA polymerase we have used a partially purified preparation of the gene-5 protein that may be activated by addition of thioredoxin. Blocking of the thiol groups of thioredoxin by amino-ethylation, performic acid oxidation or carboxymethylation completely inactivates the molecule. This result suggests that either the two thiol groups are necessary for an enzymic function or that the conformations of these modified thioredoxins are altered to destroy the subunit interactions.

Another approach to understanding the function of thioredoxin in phage-T7 DNA polymerase is to analyse *E. coli* mutants (tsnC) containing a defective thioredoxin. A thorough investigation of one mutant, tsnC 7004 gave no indication of the presence of thioredoxin as analysed by immunochemical or enzymic methods, strongly suggesting that this mutant is a nonsense mutant (Holmgren et al., 1978). Two other *E. coli* mutants, 7007 and 7008 (Chamberlin, 1974) contained detectable amounts of thioredoxin in crude extracts as measured with thioredoxin reductase or by immunoprecipitation. Thioredoxin from mutant tsnC 7007 was isolated in homogeneous form and characterized. This mutant tsnC 7007 thioredoxin was more acidic than wild-type thioredoxin and peptide 'mapping' and dansyl–Edman degradation showed that the protein had a replacement of glycine-92 by an aspartic acid residue. The purified mutant tsnC 7007 protein was inactive when assayed with gene-5 protein in a DNA polymerase assay. It retained decreased activity as substrate for thioredoxin reductase and ribonucleotide reductase which thus allowed a differentiation between the electron-transfer function and the ability to reconstitute phage-T7 DNA polymerase. The substitution of glycine-92 by an acidic residue occurs in the peptide chain preceding the C-terminal α-helix. It seems highly likely that the change in conformation of thioredoxin in this
region will not allow the correct subunit interactions in the reconstituted phage-T7 polymerase necessary for a functional enzyme. The analysis of further mutants may help to define the function of thioredoxin in the phage-T7 polymerase and the reason for its incorporation in this phage-induced enzyme.

This investigation was supported by grants from the Swedish Medical Research Council (projects 13X-3529 and 13P-4292), The Swedish Cancer Society and Magnus Bergvalls Stiftelse. The excellent technical assistance of Miss Marianne Dannbeck is gratefully acknowledged.


The Function and Structure of the Protein-Synthesis Elongation Factors Tu and Ts in Phage-Qβ Replicase

THOMAS BLUMENTHAL, STANLEY BROWN, RICHARD A. YOUNG and LESLIE STRINGFELLOW

Department of Biology, Indiana University, Bloomington, IN 47401 U.S.A.

The small, single-stranded-RNA-containing phages of Escherichia coli (Qβ, R17, f2 MS2) contain only enough genetic material to code for three or four polypeptide chains. Yet they must perform many of the functions performed by much larger viruses; namely translation and replication, gene control, encapsidation, and cell lysis. They have been shown to accomplish this by a series of economies including multiple use of phage-coded proteins, use of genome secondary structure for gene control, translation of the same genetic material to produce two different proteins, and utilization of host proteins for apparently novel purposes. We shall describe an example of the last of these; namely the adoption of the host protein-synthesis elongation factors Tu and Ts for the purpose of replication of phage-Qβ RNA.

The enzyme responsible for catalysing this replication reaction, phage-Qβ replicase, was purified to homogeneity by Kamen (1970) and by Kondo et al. (1970), and was found to be composed of four non-identical polypeptide chains. Only one of these is coded by the phage. The other three are host proteins and have subsequently all been shown to be derived from the protein-synthetic machinery. They are 30 S ribosomal-protein S1 (Wahba et al., 1974; Inouye et al., 1974) and the protein-synthesis elongation factors Tu and Ts (Blumenthal et al., 1972).

In the present paper we shall describe experiments designed to determine whether the functions that the elongation factors perform in phage-Qβ RNA replicase are derived from those they are known to perform in protein biosynthesis.

Are the known protein-synthetic activities of the elongation factors involved in the RNA-synthetic reaction of phage-Qβ replicase?

It is not immediately obvious how proteins normally responsible for catalysing transfer of aminoacylated tRNA molecules to ribosomes might be involved in RNA