# AN APPARENT RE-ORIENTATION OF PROTEIN MONOLAYERS BY NUCLEIC ACID

by

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It was reported in 1946[1] that yeast ribonucleic acid (RNA) caused an expansion of haemoglobin monolayers when present in the substrate, evidently owing to the formation of a surface RNA-protein complex. The increasing evidence that RNA plays a fundamental rôle in protein synthesis[2] and that this rôle may be of a catalytic nature[3], has made a re-examination of the phenomenon desirable. The following observations have been made with a LANGMUIR surface balance, using 0.01 *M* sodium potassium phosphate buffer (pH 6.8) as substrate. The spreading solution was a solution of heat-denatured horse haemoglobin (0.5 mg per ml) in 60 % *iso*propyl alcohol, 0.4 *M* with respect to sodium acetate.

The expanding effect, although it reaches completion almost instantaneously, is nevertheless dependent upon the nucleic acid concentration in the substrate. This suggests that the surface complex is in a state of equilibrium with the underlying RNA.

When film potential measurements are made during the compression by means of an ionized air gap, and the surface dipole moments of the protein molecules are calculated, neglecting a possible contribution to the potential by the RNA, it is found that the dipole moment-area curves for RNA-haemoglobin and haemoglobin meet at an area of about 0.82 $m^2$ per mg protein and follow virtually identical courses at smaller areas (Fig. 1).

The area 0.82 mg per mg corresponds to the area of minimum compressibility of the protein film as calculated from the pressure-area curve, and presumably to a close packing of the polypeptide chains in the surface[4]. That the nucleic acid is still associated with the protein at this area, is shown by the fact that it continues to exert an effect upon the surface pressure (Fig. 1). Since the surface dipole moment is an expression of the orientation of the dipoles in the film, a reasonable interpretation of the phenomenon appears to be the following:

The nucleic acid enters into combination with the protein by hydrogen bonding. The bonds may arise between the peptide linkages and the appropriate centres on the purine and pyrimidine rings. The X-ray data of ASTBURY[5] show that the intramolecular spacings of RNA and protein are well suited to such an effect. The polypeptide chains, bearing the highly charged nucleic acid, will experience strong mutual repulsion, and it is to be expected that the pressure-area curve will be displaced towards higher areas in relation to that of the protein in the absence of RNA. Compression of the film will nevertheless tend to bring about a close packing of the polypeptide chains. When this is achieved, the surface pressure must clearly be greater than in the absence of RNA, owing to the high charge still borne by the chains.

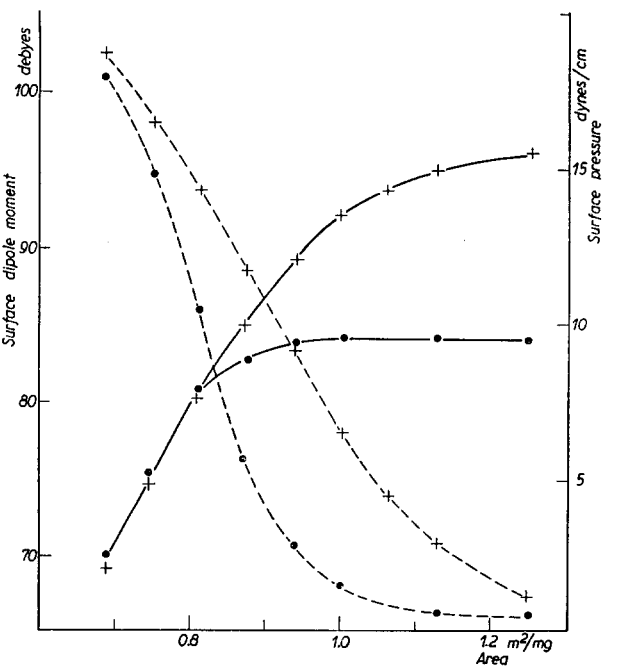


Fig. 1. Pressure-area curves (broken) and dipole moment-area curves (full) for haemoglobin monolayers on 0.01 *M* phosphate buffer (pH 6.8) alone (dots) and in the presence of 90 mg yeast RNA per litre (crosses) The dipole moments are expressed in debyes per molecule of protein.

Since it fails to show any effect at high compressions, it appears unlikely that the hydrophilic nucleic acid will contribute materially to the asymmetry potential across the film at any area. The form of the dipole moment-area curves therefore suggests that the nucleic acid, at areas greater than that representing close packing, causes a displacement in the orientation of the protein dipoles, in relation to that assumed normally at a water surface.

Since a precisely analogous effect is obtained when the haemoglobin is replaced by poly-DL-leucine (mean molecular weight $> 25{,}000$), the dipoles concerned are probably those associated with the peptide bonds. In other words, the whole polypeptide framework would seem to have undergone re-orientation in such a manner that the sum of the vertical components of the dipole moments is increased.

The predominance of hydrogen bonding between the protein and the nucleic acid is also suggested by the effect on poly-DL-leucine, and confirmed by the finding that the effect of RNA on haemoglobin is almost completely eliminated when urea is added to the substrate to a concentration of 2%.

Deoxyribonucleic acid from thymus gland has been found to give effects similar to those of RNA.

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# THE ACTIVE SURFACE OF PSEUDO-CHOLINESTERASE AND THE POSSIBLE ROLE OF THIS ENZYME IN CONDUCTION

by

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Whereas the function of true cholinesterase in the conductive process is indicated by the specific localization of this enzyme[1], little success has so far attended all attempts to define the physiological function of pseudo-cholinesterase[2]. The affinity of this enzyme for choline esters is not yet sufficiently understood, since ADAMS AND WHITTAKER[3] on the basis of their experiments considered it doubtful whether "a negative, nitrogen-attracting group exists in the plasma enzyme".

We have now applied to the pseudo-cholinesterase of human plasma the same criteria that led us previously to a definition of the active surface of true cholinesterase and to a possible explanation of its role in the process of nerve conduction[4,5,6]. We find that quaternary ammonium ions form a typical series of inhibitors, in which—in analogy to their affinity for cation exchangers—the effect is proportional to chain length. However the "limiting" size of the ion appears to be much greater than for the true esterase. In Table I we compare the $I_{50}$-values for both enzymes. Our figures show clearly that the pseudo-esterase contains a negative site in the neighborhood of the esteratic site. This conclusion finds further support in the observation that the inhibitory effect of eserine, a tertiary base, shows a similar pH dependence as was found previously for the system eserine—true esterase[7].

It is especially noteworthy that hexamethonium, which does not reveal any effect towards the true esterase, is an effective inhibitor of the pseudo-esterase, in view of the fact that this enzyme is present in various parts of the nervous system[8]. The ganglionic blocking action of hexamethonium and other quaternary ammonium salts[9] may be related to the presence of pseudo-cholinesterase in ganglionic synapses, where the enzyme could play a similar role in conduction as does the true esterase in other parts of the nervous system[10]; but due to its smaller turnover number the pseudo-