tracks F, G and H). When re-initiation of protein synthesis was prevented by the addition of ecdie (10 µM) after 1 min, it was found that conversion of pretrypsinogen into trypsin decreased after 5 min of translation.

Translation experiments in vitro have shown that passage of secretory proteins across the endoplasmic-reticulum membrane is coupled to translation, and that processing is not possible if microsomal vesicles are added after translation has terminated (Blobel & Dobberstein, 1975). Re-initiation of protein synthesis from dog pancreatic mRNA was prevented by the addition of edeine after 1 min of translation, and samples were taken at various time intervals thereafter and further incubated in the presence of membranes at a final concentration of 6.54 260 units/ml. Maxim: protection to exogenous trypsin and chymotrypsin (300 µg/ml) occurred when membranes were added within 2 min of the start of initiation, whereas little resistance was observed after 5 min, suggesting that translocation was no longer possible.

Cleavage of pretrypsinogen by enterokinase decreased after 5 min of translation, when only the N-terminal section of the chain would have been completed (Lingappa et al., 1979). Processing of nascent pretrypsinogen by microsomal membranes is limited to the first 2 min of translation. Thus translocation and concomitant processing by signal peptidase occurs in an analogous manner to the cleavage of the nascent chain by pretrypsinogen after enterokinase. The observed lack of post-translational processing may therefore be the result of the propensity of the signal sequence to take part in the formation of secondary structure in the interior of the completed pre-protein (Austen, 1979), as this would prevent any interaction with components of the endoplasmic-reticulum membrane involved in transport, or with enterokinase.

X-ray crystallographic studies have shown the activation sequence of trypsinogen to be on the surface of the molecule and therefore readily accessible to proteinases (Fehlhammer et al., 1977). In pretrypsinogen, the activation sequence is not accessible and, although signal-peptidase cleavage is not always required for secretion to take place (Lingappa et al., 1979), it may generally be required to allow the protein to fold into a biologically functional form.

We gratefully acknowledge the support of the Wellcome Trust and a gift of human enterokinase from Dr. David Grant.


The incorporation of glycophorin into phospholipid liposomes: effect of acyl chain length on lectin agglutination

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The incorporation of the major sialoglycoprotein, glycophorin, into phospholipid liposomes has been investigated by a number of workers (Grant & McConnell, 1974; MacDonald & MacDonald, 1975; Brület & McConnell, 1976; Sharam et al., 1977, Van Zoelen et al., 1978a,b; Gerritsen et al., 1980; Goodwin & Jones, 1980). In unilamellar liposomes glycophorin withdraws lipid from participation in the gel-lamellar phase transition and the number of non-participating lipid molecules per glycophorin molecule increases from approximately 50 to 250 on increasing the acyl chain length from C14 to C18 in the diacylphosphatidylcholines (Goodwin & Jones, 1980).

On incorporation of glycophorin into the bilayers of liposomes they become susceptible to agglutination by WGA which has binding sites for the N-acetylneuraminic acid residues in glycophorin (Blavatanan & Katlic, 1979). To assess the role of bilayer fluidity on the agglutination process the agglutination of sonicated liposomes has been studied over a range of temperature for a homologous series of diacylphosphatidylcholines with acyl chain lengths of 14, 16 and 18 carbon atoms. The gel-lamellar phase transition temperatures (Tg) for these synthetic materials were found by differential scanning calorimetry to be 24.5, 42.5 and 55°C respectively.

Glycophorin was isolated from human erythrocyte membranes by the method of Marchesi & Andrews (1971); no attempt was made to separate the different glycophorins (Furthmayr, 1978).

Unilamellar liposome dispersions were prepared at a lipid concentration of 0.02% with respect to buffer and glycophorin:lipid molar ratio of 2.0 × 10⁻³.

Agglutination rate was assessed by measuring spectrophotometrically the rate of increase in turbidity on addition of WGA. Liposomes (0.6 cm³) were added to each of a pair of 1 cm³ quartz cuvettes. After temperature equilibration in a Cary 219 spectrophotometer agglutination was initiated by the addition of 60 µl of WGA (1 mg·cm⁻³ in buffer, pH 7.4) to the sample cuvette and an identical volume of buffer to the reference cuvette. The absorbance was recorded at 340 nm up to 500 s from mixing. The absorbance–time plots were fitted to orthogonal polynomials and the initial rates of change of absorbance (agglutination rate) (dA/dt) were computed.

Fig. 1 shows the agglutination rate for glycophorin-containing liposomes as a function of temperature from which it is seen that the rate decreases with increasing temperature and shows a transition in the region of the onset of chain-melting of the bilayer phospholipid. The rates below Tg increase in the acyl chain sequence C14 < C16 < C18. These experiments were done at intermediate glycophorin:lipid ratio and WGA concentration (agglutination rate increased with the molar ratio of glycophorin:lipid in the range 1 × 10⁻³ to 4 × 10⁻³ and with WGA concentration reaching a limiting value at a concentration of 7 µM) so that neither of these factors were sufficiently high to give the maximum agglutination rates observable in these systems. Agglutination was shown to be completely reversible by addition of N-acetylglucosamine, showing that no significant fusion of liposomes occurred.

A decrease in rate with increasing temperature implies a negative activation energy, i.e. no potential barrier to agglutination, and has been found in other liposome systems (Rando & Bangert, 1979; Slama & Rando, 1980). However, although bilayer fluidity clearly plays a role in the agglutination process, other factors may contribute to the decreasing rate of
agglutination with \( T_c \). It is probable that glycophrin is clustered in the bilayer below \( T_c \). (Grant & McConnell, 1974; Chapman et al., 1979; Gerritsen et al., 1980) and the shorter the acyl chain length the fewer the number of disordered (i.e. those not participating in the phase transition) lipids per glycophrin molecule in the clusters; hence it would seem that for the shorter acyl chains, when WGA binding and bridging between liposomes is restricted by steric factors, the agglutination rate is smaller. Should WGA binding become stronger with increasing temperature there would be fewer free binding sites in the clusters available for bridging and the agglutination rate would decrease. Microcalorimetry measurements indicate that the enthalpy of interaction of WGA with liposomal glycophrin is indeed endothermic for each lipid system. For dipalmitoylphosphatidylcholine liposomes it amounts to 570kJ mol\(^{-1}\) of WGA. Thus WGA binding will increase with temperature. Above \( T_c \), the glycophrin clusters will break-up and the rate of agglutination should be much less dependent on the acyl chain length, as is observed.


Studies on lipid peroxidation and its effect on the clotting activity of a commercial preparation of thromboplastin

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Tissue thromboplastin (Factor III) is a membrane-bound lipoprotein that interacts with Factor VII in the presence of Ca\(^{2+}\) and initiates the extrinsic pathway of clotting. Thromboplastin is released from its latent state to initiate this pathway after tissue damage and cell-membrane disruption.

The exact nature of tissue damage is difficult to define, but prolonged trauma may also lead to eventual loss of the clotting stimulus. For instance, the work of Roushbal & Tappel (1966) and Schaic & Karel (1976) has indicated that, after oxidation of membrane lipids, reactive aldehydes such as malondialdehyde and other free-radical substances may result in covalent cross-linkages being inserted into membrane proteins. Indeed, Stevenson et al. (1980) suggested that the method of production of the British Comparative Thromboplastin from human brain, which involves maceration of the tissues, causes the breakdown