our results suggest for the apoprotein a slightly asymmetric shape. In dioxan/water (2:3, v/v), a large concentration-dependence of the kinematic viscosity is observed. This could be related to increased protein–protein electrostatic interactions in this solvent. The sedimentation coefficients, extrapolated to zero concentration, are equal to 3.9, 4.1 and 2.9 S in 1 and 6% acetic acid and in dioxan/water (2:3, v/v) respectively. From these values, some variations in the state of protein aggregation can be excluded.

Table 1 summarizes the results of the fluorimetric study. The identical values of maximum emission wavelengths for labelled apoprotein and N-acetyl-L-cysteine in organic solvent give evidence of the accessibility to the solvent of the thiol groups labelled, up to 1.5 per protein molecule. Results obtained in aqueous solution can be interpreted as a burying of the labelled thiol groups inside the protein, the more reactive groups in organic solvent being the more deeply buried or immobilized in aqueous solvent.

All these results are consistent with the existence of large structural differences between the apoprotein in pure organic solvent and in aqueous solutions, whereas no difference in the state of aggregation has been evidenced in the aqueous solution, unless an organic co-solvent is added at a concentration of 40%.


Lipoprotein Complexes between Glucagon and Dimyristoylglycerophosphocholine (Dimyristoyl Phosphatidylcholine)

ANDREW J. S. JONES and RICHARD M. EPAND

Department of Biochemistry, McMaster University, Hamilton, Ont. L8S 4J9, Canada

Glucagon has an amino acid sequence which would allow it to form amphipathic helices (Epand et al., 1977), and such structures have been observed in the crystalline form of the hormone (Blundell et al., 1976). Interaction of lipids with proteins capable of forming amphipathic helices has been demonstrated for the human (Assmann & Brewer, 1974) and porcine (Andrews et al., 1976) high-density lipoproteins, a lipoprotein from the outer membrane of Escherichia coli (Inouye, 1974) and apolipoprotein C-III (Pownall et al., 1974; Novosad et al., 1976) and amyloid A (Segrest et al., 1976).

When a suspension of glucagon is vortexed in a tube coated with a thin film of 1,2-dimyristoyl-sn-glycerol-3-phosphocholine at 40°C in 0.1 M-acetate (adjusted to pH 7.4 with NH₄OH) and spun at 40000g for 20 min at 5°C, the resulting supernatant contains essentially all of the lipid. The excess of glucagon in this solution over a saturated glucagon solution under the same conditions depends on the amount of lipid initially present and represents a molar ratio of lipid to glucagon of 35:1 (Jones et al., 1977). The complex can be formed under widely differing ionic-strength conditions (0.01 M-ammonium acetate, pH 7.4, or in 0.5 M-phosphate adjusted to pH 7.4 with KOH + 0.15 M-NaCl) and is independent of pH in the range 6–9.

The peptide has no effect on the protein nuclear-magnetic-resonance spin lattice-relaxation times (T₁) of the choline methyl or fatty acid methylene resonances, but causes
a marked broadening of the methylene and terminal methyl resonances. All the choline methyl resonances are detected and are accessible to the externally added paramagnetic broadening reagent Mn²⁺. The phase transition of the lipid (approx. 24°C) is found to be unaltered by glucagon when monitored by pyrene excimer fluorescence. Differential scanning calorimetry indicates no significant change in the enthalpy of transition, although the transition is somewhat broadened by extension to higher temperatures. These data are interpreted as reflecting the presence of a non-vesicular bilayer structure in which the rate of lateral diffusion of the lipid is drastically decreased without affecting the intramolecular lipid motion.

The following hydrodynamic and other data were obtained: $s^2_{20,w} = 9.2$; $[\eta] \approx 8.0$ ml/g; $D_{20,w} \approx 1.4 \times 10^{-7}$ cm² s⁻¹; $\bar{v} = 0.920$ ml/g; mol.wt. = $2 \times 10^6$; shape, flattened disc with dimension approx. 7.5 nm x 30 nm (negative staining and freeze-fracture electron microscopy).

The u.v. difference spectrum of the complex against glucagon and the fluorescence characteristics (approx. 3-fold increase in tryptophan quantum yield with a blue shift of 12 nm; Epand et al., 1977) are indicative of a hydrophobic environment of the tryptophan and tyrosine residues in the complex. The temperature-dependence of the u.v. thermal difference spectrum shows a discontinuity around the 'pre-melt' temperature of the lipid (14-15°C), a phenomenon not shown by model peptides (Nicola & Leach, 1976). The increased intensity of the aromatic c.d. (circular dichroism) bands also shows a discontinuity at the 'pre-melt' temperature (see Fig. 1). These latter two observations

---

**Fig. 1. Effect of temperature on the fractional change in the magnitude of aromatic circular-dichroism bands of the glucagon-dimyristoylglycerophosphocholine complex in 0.1 M-ammonium acetate, pH 7.4**

$\phi$ is the fractional change at temperature $T$ defined as $\left( [\theta]_T - [\theta]_6 \right) / \left( [\theta]_6 - [\theta]_{20} \right)$ where $[\theta]_T$, $[\theta]_6$ and $[\theta]_{20}$ are the mean residue ellipticities at temperatures $T$, 6° and 20°C respectively. Vertical bars span approx. 90% of the noise level from 5 min continuous observation; lateral bars represent the temperature change during that period. Ellipticities were measured at 296 nm (△), 292 nm (○), 285 nm (□), 280 nm (▽) and 272 nm (■).
demonstrate that the conformation of the peptide is sensitive to this minor thermal transition. However, the monotonic decrease in intensity of the peptide-bond c.d. bands with increasing temperature shows that the 'pre-melt' of the lipid does not affect the gross conformation of the molecule.

At temperatures above the main lipid phase-transition temperature (i.e. >23°C) the complex is unstable and precipitates both the lipid and excess glucagon from solution and the interactions described above are abolished. If the solution is recooled to below the phase-transition temperature the complex re-forms in a totally reversible manner. When 1,2-dipalmitoyl-sn-glycero-3-phosphocholine is used the same phenomena are observed but the complex formed is stable up to 40°C (the phase transition of this lipid is 41°C).

The functional aspects of this phenomenon require investigation. These include the possibilities of serum lipoprotein involvement in glucagon action and the control of target-cell sensitivity to the hormone by regulation of plasma-membrane fluidity.

This work was supported by National Research Council (Canada) grant number A-9849.


---

**Protein-Mediated Transfer of Phosphatidylcholine to Myelin**

**ERIC M. CAREY and PETER C. FOSTER**

*Department of Biochemistry, University of Sheffield, Sheffield S10 2TN, U.K.*

From experiments *in vivo* with radioactive precursors of phospholipids, the different components of myelin phosphatidylcholine (the phosphate, glycerol, fatty acyl and cholino moieties) are replaced at significant though different rates (Horrocks *et al.*, 1976). Since isolated myelin possesses no biosynthetic capacity, the bulk of the myelin phospholipids must be metabolized by replacement of the whole phospholipid molecule in the myelin membrane through exchange of individual phospholipid molecules, or through membrane vesicle transfer, or metabolized while the phospholipid is still present in the plasma membrane of the myelinating cell and in contact with cell cytoplasm and endoplasmic reticulum. Experiments have been carried out to examine whether myelin phosphatidylcholine can be replaced by exchange with other membrane pools of phosphatidylcholine by a process involving soluble phosphatidylcholine-exchange proteins.

Myelin was isolated from adult rat brain and bovine white matter after tissue homogenization in 0.3m-sucrose/0.1m-EDTA/10m-Tris/HCl (pH 7.2) (Suzuki *et al.*, 1967). Osmotically shocked myelin (Agrawal *et al.*, 1970) was free of NADPH–cytochrome c reductase, 5'-nucleotidase, cytochrome c oxidase and acetylcholinesterase activities, but possessed high 2':3'-cyclic adenosine monophosphate 3'-phosphohydrolase activity. Electron microscopy revealed numerous multilamellar membrane structures, frequently with separations between adjacent lamellae, and individual membrane fragments, some as closed vesicles.

1977