

The Solubility of Proteins at High Ionic Strengths in the Presence of Dextran

Torvard C. Laurent

Department of Medical Chemistry, University of Uppsala, Sweden

The presence of dextran decreases the solubility of albumin, γ -globulin fibrinogen, and α -crystallin at high ionic strengths. The relative effect increases with increasing size of the protein but is independent of the degree of polymerization of dextran, the pH, the absolute salt concentration or the absolute protein concentration. The results can be discussed in terms of a large effective volume of dextran. The importance of the observations for natural polysaccharide-protein systems will be emphasized.

The Incorporation of Phosphorus into Human Erythrocyte Ghost Fractions

Gunnar Ågren

Institute of Medical Chemistry, University of Uppsala, Sweden

The entry of phosphorus into the red cell is not a simple diffusion process. However, phosphate is taken up by the red cell, and since the content of cell inorganic phosphate does not seem to increase there must be an equal source of loss from the cell. Since no exchange between internal and external orthophosphate can be demonstrated it would appear that both uptake and loss of phosphate is linked to cell metabolism presumably through the breakdown of phosphate esters occurring in close connection with the cell surface¹. The observation that labelled phosphopeptides and phosphorylserine can be isolated from the protein fraction of red cell ghosts incubated with labelled orthophosphate (³²P_i) is therefore of considerable interest^{2,3}. Newer investigations have given the following additional results.

The ghosts were washed six times with distilled water saturated with CO₂ and again six times with 2×10^{-2} M tris-glycylglycine buffer, pH 8.2, in the Spinco ultracentrifuge. The washings concentrated by ultrafiltration⁴ as well as the ghost residues suspended in a corresponding volume of buffer were incubated for different times with ³²P_i at pH 7.4. From 60 to 90 % of the ³²P_i-incorporating activity was present in the ultrafiltrate in the form of phosphorylserine, phosphopeptides and some other unidentified fractions isolated from the hydrolysates of the Schneider protein fractions.

The phosphopeptides and phosphorylserine were recovered in the first four column volumes of eluate (0.01 N HCl) from the Dowex 50 columns. When the elution proceeded labelled peaks were also found in positions corresponding to those of phosphorylethanolamine and phosphorylcholine⁵. The rate of ³²P_i-incorporation into the substances from these column fractions was much slower and maximal incorporation of radioactivity seemed not to be reached even after 16 hours of incubation. The results are not in accordance with the present view that the lipids in the mature red cell are static⁶.

The solubilized labelled protein fraction from the red cell ghosts still contaminated by hemoglobin was put through a column of cross-linked dextran (Sephadex G 200). A colourless labelled protein was recovered from the column well separated from the unlabelled hemoglobin peak. It would seem that the protein complex which incorporated ³²P_i has a rather high molecular weight. A comparison indicated that the degree of labelling of the solubilized protein fraction and the ghost residues was not changed after storage for one week or more at +4°C, but considerably diminished at -16°C even for shorter periods.

1. Jones, H. and Gourlay, D. R. H. *Biochim. Biophys. Acta* **14** (1954) 335.
2. Ågren, G. and Engström, L. *Acta Chem. Scand.* **10** (1956) 876.
3. Ågren, G., Hallberg, B. and Ronquist, G. *Acta Chem. Scand.* **16** (1962) 1770.
4. Berggård, J. *Arkiv Kemi* **18** (1961) 291.
5. Ågren, G. *Acta Soc. Med. Upsalien.* **67** (1962) 55.
6. Pranker, T. A. J. *The Red Cell*. Blackwell Scientific Publications, Oxford 1961.