On the Presence of Phosphoproteins in Erythrocyte Ghosts

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In a previous paper it has been demonstrated that the main part of the phosphoproteins in *E. coli* cells are located in the cell wall preparations [1]. It was therefore of interest to carry out a similar investigation with some animal cells. The erythrocytes seemed to offer a suitable material. This paper deals with the isolation of phosphoserine from erythrocyte ghosts.

After some preliminary work with earlier methods the ghosts were finally prepared in the following manner. 500 ml samples of blood were washed with cold physiological sodium chloride solution at least twice in a centrifuge taking 4 liters. After each washing the leucocytes were removed by decantation and suction. 3 volumes of cold distilled water were added. After haemolysis the remaining leucocytes were removed by centrifugation in a Spinco centrifuge with rotor 21 at 6 000 r.p.m. for 20 min. The suspension of erythrocyte ghosts was decanted and centrifuged for 20 min. at 18 000 r.p.m. in the same rotor. The voluminous precipitate was washed once with distilled water and at least 3 times with a 0.1 % sodium chloride solution.

The ghosts were suspended to a final volume of 100 ml in 0.1 M Tris (tris-(hydroxymethyl)aminomethane) at pH 7.4, and in the presence of 0.01 M $MgCl_2$. 5 mC ^{32}P was added and the incubation was finished after different times by the addition of trichloroacetic acid to a final concentration of 10 %. Lipids and nucleic acids were removed as previously described [2]. The residue from this treatment, taken as the Schneider protein fraction weighed about 2 g. This fraction was partially hydrolysed according to Lipmann [3]. Radioactive phosphoserine could be isolated both from pig and human erythrocyte ghosts in the same manner as previously described [4].

A ghost suspension was quickly heated to and kept at 80° C for about 1 minute, followed by rapid cooling. After incubation with ^{32}P it was not possible to detect more than traces of activity in the isolated phosphoserine fractions. A similar result was obtained when the ghosts were incubated with ^{32}P in the presence of monoiodoacetate to a final concentration of 0.01 M.

For the determination of the specific activity the effluents from the Dowex 50−8X columns were run through Dowex 1−X2 columns according to Busch *et al* [5]. The specific activity of the pure phosphoserine isolated from pig erythrocyte ghosts incubated with ^{32}P for 20 min. at room temperature was 3 550 cpm per μg P. It may be of interest that the specific activity of the pure phosphoserine isolated in earlier experiments [7] from rat liver fractions was about 4 500 cpm per μg P.

The trichloroacetic acid centrifugate from the reaction mixtures of erythrocyte ghosts and ^{32}P were run through Dowex 1−X2 formate columns according to Siekevitz and Potter [7]. Several peaks of radioactivity could be observed, but so far they have not been identified. Ultraviolet absorbing peaks could not be found in the eluates.

These experiments demonstrate that the ghosts contain phosphoproteins which may be active in the phosphorus metabolism of the erythrocytes. A metabolic pontential of erythrocyte ghosts has recently been recorded by Lionetti [8]. He used ghost plasma suspensions and studied the effect of adenosine on plasma inorganic phosphorus.

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