CHANGES IN MEMBRANE CONSTITUENTS DURING DIFFERENTIATION OF RABBIT RETICULOCYTES

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1. Introduction

Maturation of reticulocytes into erythrocytes is accompanied by a loss of the mitochondrion [1], a complete cessation of RNA synthesis [2], a progressive degradation of the polyribosomes and a loss of at least one of the initiation factors required for protein synthesis [3].

One of the most striking differences, however, between the highly active reticulocyte and the more passive erythrocyte is the change which occurs at the level of the cell surface as shown by labelling with cationized Ferritin [4].

The constitution of the reticulocyte membrane is not as well known as that of the erythrocyte membrane and it was tempting to relate the changes at the cell surface level with more intimate differences in the membrane constituents. On the other hand, as our group is working with reticulocytes as a model for interaction between membranes properties and protein synthesis [5] it was essential to know more about the reticulocyte membrane. For this purpose we decided to investigate the composition of the cell membrane from different age groups of reticulocytes separated by differential floatation through phtalate oil, and to compare the gel electrophoresis obtained to those obtained from mature erythrocyte membranes.

2. Materials and methods

Reticulocyte enriched blood was obtained by injection of phenylhydrazine to rabbits. Reticulocytosis was checked by the brilliant cresyl blue method.

Reticulocytes of three age groups, old, medium and young, were obtained by differential floatation through phtalate oil following the procedure of Danon et al. [6]. Isolated cells were washed in isotonic buffer (RBC) containing NaCl 150 mM, $MgCl_2$ 5 mM, Tris–HCl pH 7.4 10 mM, and then lysed by hypotonic choc. Hemoglobin poor membranes were obtained by repeated hypotonic choc followed by resuspension in isotonic buffer [7]. After centrifugation for 5 min at 6000 *g* pellets were treated with SDS buffer according to Laemlli's [8] procedure to obtain a good separation of polypeptide chains.

Slab gels presenting three layers of acrylamide were polymerised, and samples were layered on wells and run for 3 hr at 120 V. Slab gels were stained with Coomassie blue. Patterns obtained were run for Absorbance measurement in a Gilford densitometer.

3. Results

In a three-layered polyacrylamide gel as shown in fig.1, the high molecular weight proteins are clearly visible and were numbered according to accepted nomenclature [9]. Several differences appear immediately in comparing the pattern obtained from young reticulocyte and mature erythrocyte. Particularly, bands 2-1 and 2-2 are replaced in the young reticulocyte by band R-1 and a very faint doublet R-2,1 and R-2,2. Band 3 from the erythrocyte is replaced by bands R-4 and R-5 with their minor satellites R-3 and R-5,1. Below band 4-2 one can distinguish a minor erythrocyte band which is much stronger in young reticulocytes and is labelled R-6. As far as low mol. wt polypeptides are concerned, they are

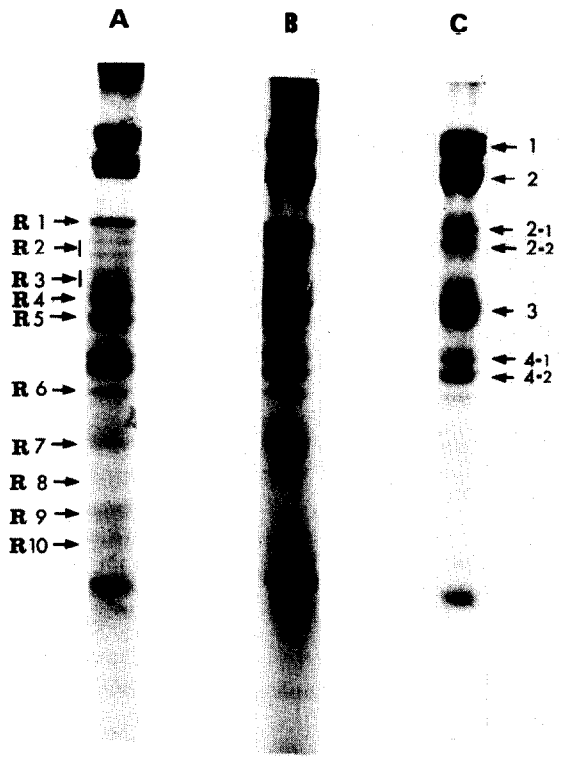

Fig.1. SDS polyacrylamide gels of reticulocytes and erythrocyte membranes. Membranes from reticulocytes of two age groups and erythrocytes were prepared as in Materials and methods and treated with SDS and mercaptoethanol. 55 μg samples were run on a composite gel containing three layers of respectively 3%, 5% and 10% acrylamide and stained with Coomassie blue. One can clearly see the bands labelled R_n on the young reticulocyte sample (a) which have no correspondance with the erythrocyte bands on gel (c). On the contrary, gel (b) from old reticulocytes shows a resemblance both with gel (a) and with gel (c).

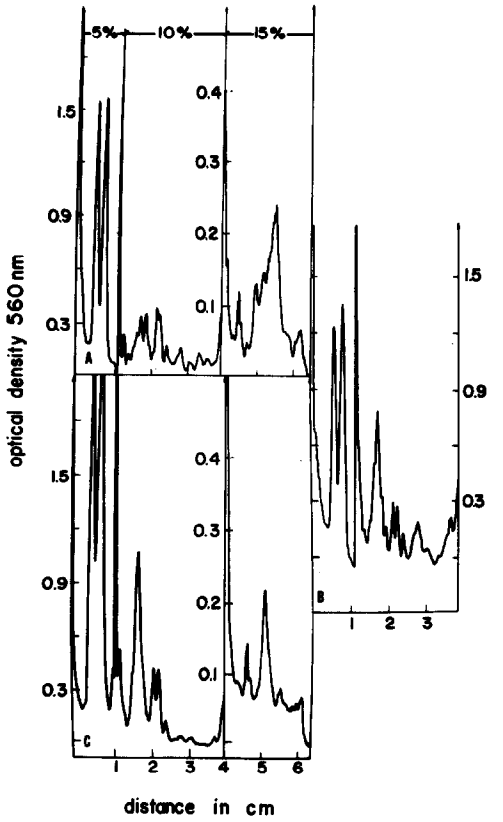

Fig.2. Densitometer tracing of gels from reticulocytes and erythrocytes membranes. In this figure a 3%, 5% and 10% gel was traced using a microdensitometer. The same sample was also run on a 10%, 15% gel where resolution for the low mol. wt region was improved. The two graphs were put together and aligned for a recognizable peak for young reticulocytes (a) and erythrocytes (c). For old reticulocytes (b) only the 5% and 10% gel was used.

demonstrated better on 15% gels and their tracings on fig.2 show that here again there are multiple differences between young reticulocytes and erythrocytes and particularly bands R-7 and R-9.

When comparing the patterns of young reticulocytes, old reticulocytes and erythrocytes it is possible to follow a 'differentiation' pattern where the R-1 and R-2 region in gel a) get thicker in gel b) to form the doublet which are bands 2-1 and 2-2 in gel c). An opposite picture is obtained with bands R-4 and R-5 which form progressively the unique major band 3 in gel (c). The low mol. wt bands tend to disappear with maturation.

The differences pointed out here are only the major changes and we are currently working on the identification of the minor bands which appear solely either in erythrocytes or in reticulocytes. The fact that reticulocytes possess more polypeptides in its membrane is not surprising as the cell has to express more functions than the erythrocyte. However the presence of new bands in the erythrocyte membrane, like band 3, for instance, is surprising if one remembers that there is no new mRNA formed and that the maturation takes place with a given stock of genetic information. This could be interpreted in two ways, either a de novo translation of a previously suppressed message

existing in the reticulocyte or the presence of a very specific protease which would modify polypeptides already present in the membrane. The possibility of a contaminant like for instance ribosomes, is very unlikely at least in the high molecular weight region and indeed have been ruled out by others [10]. On the contrary we were able (Wreschner, Foglizzo and Herzberg, in preparation) to show a labelling of the membrane by incubating cells in a labelled amino acid medium for a period as short as 40 min, showing, as was also demonstrated by Lodish [11], that reticulocytes are active in synthesis of membrane proteins.

Differences in membrane composition of red cells have been recently demonstrated in erythrocytes kept for different periods of time [12] but in this case, of course, there could not be any synthesis of new components as it seems to be the case during the differentiation of reticulocytes into erythrocytes.

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