PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 167, 218–219 (1981)

Heat-induced Electrophoretic Anomalies of Red Cell Membrane *Non*sialoglycoproteins (41152)

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Abstract. The effects of heating at 100° in the presence and absence of dithiothreitol has been examined in SDS-solubilized ghost membranes and isolated integral membrane proteins from human red cells. A significant increase in electrophoretic mobility and broadening of the major polypeptide band 3 was found. The anomalous behavior of band 3 alone could explain the apparent disappearance of band 4 which has occasionally been reported in hereditary spherocytosis red cells.

Anomalous sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) behavior has been reported for a variety of membrane proteins. Factors implicated have included the amount of SDS bound, the incompleteness of disulfide reduction, and the effect of heat. The interconvertibility of the major red cell membrane sialoglycoproteins due to heating in SDS solutions is now well established (1–3). To our knowledge, there are no reports on the heat-modified electrophoretic behavior of red cell membrane *non*sialoglycoproteins.

In 1974, two groups reported the absence of band 4.2 (nomenclature of Steck (4)) in membranes derived from hereditary spherocytosis (HS) red cells (5, 6). Subsequently, various reports appeared which could not confirm the finding (7, 8). In particular, Boivin and Galand (7) pointed out that the disappearance of band 4.2 could be explained by the use of 1.0% instead of 0.1% SDS–gels. We recently examined HS cells and observed only one electrophoretic band, as previously reported, immediately below the major integral membrane polypeptide, band 3, instead of two bands, 4.1 and 4.2. This was due to having exposed our SDS-solubilized samples to 100°, a routine measure common in ghost membrane studies designed to inactivate any leukocyte-derived proteases potentially present. As a result of this observation, we have examined the effect of heat on reduced and nonreduced ghost membranes as well as on a band 3-enriched integral membrane protein fraction.

Methods. Ghost membranes from fresh human blood were prepared and analyzed electrophoretically essentially in accordance with the procedure of Fairbanks *et al.* (9). The main modifications included the use of 8 *M* urea in the gels and a 20:1 monomer-to-crosslinker ratio. Heat treatment in this paper refers to exposure to 100° for 10 min. Unheated samples were incubated at 37° for 30 min. The reductant, when used, was 0.6% dithiothreitol (DTT). The term nonsialoglycoproteins as used in this report refers to those membrane proteins stained by Coomassie blue in contrast to those stained by periodic acid–Schiff reagent (sialoglycoproteins). We recognize that band 3 contains a low amount of sialic acid and that sialoglycoproteins may stain faintly with Coomassie blue. Integral membrane proteins were obtained from ghost membranes by the alkaline extraction method of Steck and Yu (10). Estimations of membrane protein were based on $A_{280}^{1\%}$ = 12.0 in 0.2% SDS. This was established by comparison with values obtained in the Lowry procedure with bovine serum albumin as standard (11).

Results and Discussion. As shown in Fig. 1, there is no discernible difference between DTT-reduced and nonreduced, unheated ghost membranes (gels 1 and 2). This is somewhat unusual in that reduced, unfolded proteins are generally retarded in electrophoretic mobility. Under the same

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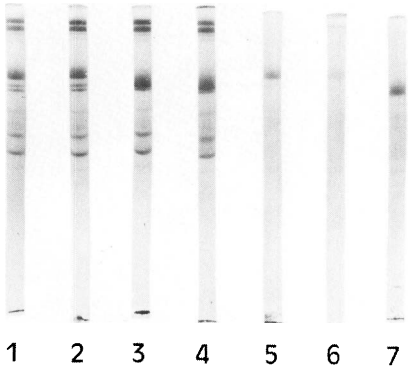

FIG. 1. SDS–PAGE of ghost membranes and integral membrane protein fraction. Gel 1: Unheated, reduced ghosts; gel 2: unheated, nonreduced ghosts; gel 3: heated, reduced ghosts; gel 4: heated, nonreduced ghosts; gel 5: unheated, reduced integral proteins; gel 6: unheated, nonreduced integral proteins; gel 7: heated, reduced integral proteins. In all gels, except number 6, the dark band at the top is an optical artefact, not aggregated protein. Sample load: ghosts, 26 μg protein; integral proteins, 17 μg protein. All gels stained with Coomassie blue.

conditions, reduced monomeric albumin shows a retardation in electrophoretic mobility of 21% when compared to the nonreduced form (data not shown). The anomalous finding in membranes probably reflects their low sulfhydryl content.

Gels 3 and 4 show the same ghost membranes after heating. In contrast to unheated preparations, reduction resulted in slightly retarded electrophoretic mobility of all the polypeptides. The important point to note, however, is that there is a general broadening of and increase in band 3 mobility in both the reduced and nonreduced samples, thus masking the 4.1–4.2 region. Often one band below 3 could be clearly discerned and since this would normally be designated as 4.1, it is possible to see how the impression of band 4.2 loss could be created. The broadening and forward migration of band 3 due to heat was more clearly apparent from inspection of the results with the integral protein fraction. As shown in DTT–gels 5 and 7, heating clearly resulted in a forward displacement and broadening of band 3 (gel 7). There was a slight band present below the origin in the unheated, nonreduced, integral protein sample (gel 6) which represented disulfide-aggregated material. The heated, nonreduced integral proteins (not shown) appeared identical to gel 7, but with slight aggregation as in gel 6.

In summary, heat-induced anomalous electrophoretic behavior of band 3 exists. While not as dramatic as the monomer–dimer interconvertibility seen in periodic acid–Schiff–stainable glycoproteins, it could be pertinent for the proper analysis of data in specific instances, e.g., HS cells.

This investigation was supported by National Institutes of Health Grant HL-23108.

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Received December 9, 1980. P.S.E.B.M. 1981, Vol. 167.