# THE MOLECULAR WEIGHT AND SUBUNIT STRUCTURE OF GLUCOSE-6-PHOSPHATE DEHYDROGENASE FROM HUMAN ERYTHROCYTES

P.COHEN and M.A.ROSEMEYER
*Department of Biochemistry, University College London, U.K.*

Received 13 July 1968

Glucose-6-phosphate dehydrogenase (G6PD; EC 1.1.1.49) catalyses the initial reaction of the pentose phosphate pathway, and thus its function is necessary in maintaining the level of the reduced coenzyme NADPH in various tissues. Human G6PD exhibits polymorphism, the structural gene being on the X-chromosome [1]. The variant forms differ from the normal enzyme in electrophoretic mobility or in catalytic activity [2,3]. While deficiencies in function do not appear to affect other tissues, most variants with low activity are associated with haemolytic anaemias, either chronic or induced by administration of drugs or ingestion of toxic substances. In the red blood cell, NADPH is used to reduce glutathione [4], which is required to preserve sulphydryl groups and so keep the cell intact.

On the basis of kinetic measurements, it has been suggested that catalysis by G6PD in the red cell and in other tissues may be subject to metabolic control [5-10]. In studying this possibility, or the extent of such control, it is necessary to know the molecular size and number of subunits of the enzyme.

Previous molecular weight measurements of the normal erythrocyte enzyme, termed Gd(+) B [2], varied between 105,000 [11] and 240,000 [12]. Yoshida concluded from the molecular weight in 4 M guanidium chloride that the enzyme contains 6 subunits [12], and from the number of tryptic peptides that the subunits were identical [13].

In the present investigation of the enzyme, the sedimentation coefficient was found to vary with the solvent conditions. The data also indicate that more than one dissociation equilibrium was occurring. It is necessary to separate these equilibria to establish the molecular size and subunit content of the protein.

Table 1
Effect of ionic strength and pH on the dissociation of glucose-6-phosphate dehydrogenase.

| Buffer | $S_{20,w}$ | $M_w$ | $M_z$ |
|---|---|---|---|
| Acetate pH 6.0 *I* 0.005 * | 9.9 | 238,000 | 245,000 |
| Acetate pH 6.0 *I* 0.05 ** | 9.8 | 208,000 | 207,000 |
| Acetate pH 6.0 *I* 0.05 ** NaCl 0.5 M | 9.0 | 114,000 | 120,000 |
| Acetate pH 6.0 *I* 0.05 ** NaCl 2 M | 5.9 | 152,000 | 170,000 |
| Phosphate pH 8.4 *I* 0.10 * | 7.1 | | |
| Tris-HCl pH 8.7 *I* 0.005 * NaCl 0.1 M | 6.7 | | |

Buffers indicated * contained 0.1 mM EDTA and ** 1 mM EDTA. All buffers were 10 $\mu$M in NADP, and 0.1% (v/v) in mercaptoethanol. Experiments were perfomed at 10°, with protein concentration approximately 2.5 mg/ml. Correction factors for $S_{20,w}$ were calculated from International Critical Tables. From ref. [12], a value of 0.731 was used for $\bar{v}$.

The enzyme GD(+) B was purified (Cohen and Rosemeyer, unpublished work) until it was homogeneous as judged by starch gel electrophoresis and ultracentrifugation. The specific activity was 180 units/mg, in agreement with the value previously reported for a homogeneous preparation [12].

At pH 6, the sedimentation coefficient (table 1) in 0.005 *I* acetate buffer, was 9.9 *S*, which is near the value reported by Yoshida (10.0 *S*) [12]. There was a gradual decrease in $S_{20,w}$ on addition of sodium chloride up to 0.5 M, followed by a steeper decrease on raising the NaCl concentration to 2 M (fig. 1). The biphasic character of the salt dependence suggests that

*North-Holland Publishing Company – Amsterdam*

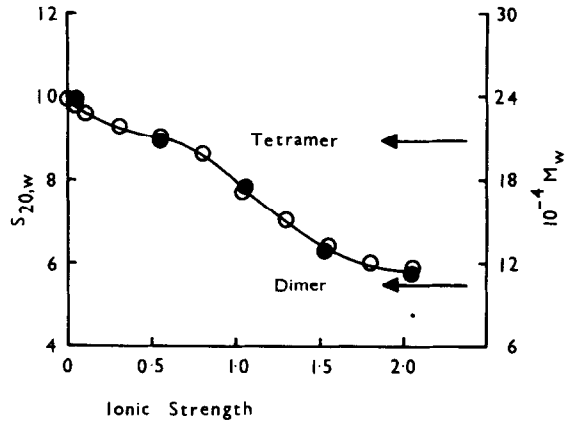


Fig. 1. Sedimentation coefficient (○) and weight-average molecular weight (●) of glucose-6-phosphate dehydrogenase. Experiments were at pH 6 and 10°C.

successive dissociation processes were occurring. It also suggests that a discrete species was present near the point at which the slope of the curve alters sharply (approximately 0.5 M NaCl), and that the protein in 2 M NaCl was approaching another discrete species.

Molecular weights determined according to the method of Van Holde and Baldwin [14] are given in table 1. It will be seen that the molecular weight in 0.5 M NaCl was near 210,000. If this value corresponds to a discrete molecular species, the higher values at low salt concentration reflect aggregation, while the lower molecular weights in high salt indicate dissociation of this molecular entity.

For an equilibrium involving two molecular species, the values observed for $M_W$ and $M_Z$ are related by the following equation [15]:

$$M_Z = (M_1 + M_2) - M_1 M_2 \frac{1}{M_W} ,$$

where $M_1$ and $M_2$ are the molecular weights of the species concerned in the equilibrium. A plot of $M_Z$ against the reciprocal of $M_W$ should give a straight line, and from the intercept and the slope the values of $M_1$ and $M_2$ may be obtained. The results for G6PD in sodium chloride concentrations between 0.5 and 2 M obeyed this relation and gave 210,000 and 105,000 for the molecular species involved in the equilibrium. Therefore, in this range, a molecule of 210,000 molecular weight dissociates to the half-molecule.

The above experiments show that in 2 M NaCl, dissociation to the half-molecule is incomplete. To test the possibility of dissociation beyond the half-molecule, the protein may be examined in denaturing solvents, or alternatively treated with reagents which promote dissociation. The latter approach has the advantage of allowing the treated material to be studied in solvent conditions similar to those used for the native enzyme. Accordingly, G6PD was allowed to react with an excess of maleic anhydride, which introduces maleyl groups at the lysine residues [16]. In phosphate buffer pH 7.0 / 0.1, the substituted protein had an $S_{20,w}$ value of 3.4 $S$ compared with 9.3 $S$ for the native enzyme in the same solvent. The treated material had an $M_W$ of 55,000 and $M_Z$ of 56,000, indicating that the preparation was homogeneous. From the lysine content [12], it is possible to allow for the increase in the molecular weight caused by the substituents. The corrected molecular weight for the protein subunit was 53,000 ± 500. The molecule of 210,000 contains 4.0 such subunits. Gel electrophoresis showed one band for the reacted material, suggesting that the subunits were either identical or very similar to each other.

Therefore, at pH 6 in salt concentration near 0.5 M, the enzyme is a tetramer of molecular weight 210,000. In high salt concentration, it dissociates to a dimer. On the other hand, in salt concentrations below 0.5 M, aggregates higher than the tetramer are observed.

The above results at pH 6 and varying salt concentration indicate the size of the enzyme, its subunit content, and the dissociation equilibria which occur. Preliminary experiments showed that the dissociation of the protein also varies with pH, the molecular weight observed decreasing with increasing pH (table 1). This would explain why a molecular weight of 105,000 was obtained for the protein in low concentration at a pH of 8 [11]. Under these conditions, the protein approaches the dimer (table 1).

Another way of dissociating the enzyme is by removal of NADP, as indicated by formation of hybrid proteins using suitable G6PD enzymes [17]. Hybridisation of two variants, Gd(+) A and Gd(-) Seattle, produced by removal and re-addition of NADP, was demonstrated by starch gel electrophoresis at pH 8.5 [18]. Since the native enzyme approaches the dimer at this pH, the formation of hybrids indicates that there was

further dissociation to the monomer on removal of NADP. The occurrence of one hybrid zone in several such experiments [17,18] suggested that each human variant was composed of identical subunits [18].

A molecular weight of 227,000 and sedimentation coefficient of 9.0 $S$ was found [19] for the variant, Gd(+) A, common among American Negroes. This variant was also thought to contain 6 subunits [19]. The experiments were performed in low salt concentration at pH 6. Thus, the $S_{20,w}$ value was lower than that of the normal protein in the same solvent, but equal to that of the normal protein in 0.5 M NaCl (table 1). Hence it could correspond to the tetrameric species, which would imply that in the variant, further associations of the tetramer in low salt do not occur. This suggests that some variants may differ from the normal protein in size owing to dissimilar association properties. Thus differences in dissociation may distinguish variants, besides the usually observed characteristics of electrophoretic mobility or catalytic function.

We are grateful to the Medical Research Council for providing a Spinco Model E analytical ultracentrifuge. One of us (P.C.) would like to thank the Science Research Council for a Postgraduate Research Scholarship.

## References

[1] D.Linder and S.M.Gartler, Am. J. Human Genet. 17 (1965) 212.
[2] World Health Org. techn. Rep. Ser. No. 366 (1967).
[3] H.N.Kirkman, P.R.McCurdy and J.L.Naiman, Cold Spring Harbor Symp. Quant. Biol. 29 (1964) 391.
[4] E.Beutler, R.J.Dern, C.L.Flanagan and A.S.Alving, J. Lab. Clin. Med. 45 (1955) 286.
[5] I.Eger-Neufeldt, A.Teinzer, L.Weiss and O.Wieland, Biochem. Biophys. Res. Commun. 19 (1965) 43.
[6] G.Avigad, Proc. U.S. Nat. Acad. Sci. 56 (1966) 1543.
[7] A.Bonsignore, A.De Flora, M.A.Mangiarotti and I.Lorenzoni, Italian J. Biochem. 15 (1966) 458.
[8] L.Luzzatto, Biochim. Biophys. Acta 146 (1967) 18.
[9] K.W.McKerns, Biochim. Biophys. Acta 121 (1966) 207.
[10] P.E.Carson, S.L.Schrier and R.W.Kellermeyer, Nature 184 (1959) 1292.
[11] H.N.Kirkman and E.M.Hendrickson, J. Biol. Chem. 237 (1962) 2371.
[12] A.Yoshida, J. Biol. Chem. 241 (1966) 4966.
[13] A.Yoshida, Proc. U.S. Nat. Acad. Sci. 57 (1967) 835.
[14] K.E.Van Holde and R.L.Baldwin, J. Phys. Chem. 62 (1958) 734.
[15] A.J. Sophianopoulos and K.E.Van Holde, J. Biol. Chem. 239 (1964) 2516.
[16] P.J.G.Butler, J.I.Harris, B.S.Hartley and R.Leberman, Biochem. J. 103 (1967) 78P.
[17] E.Beutler and Z.Collins, Science 150 (1965) 1306.
[18] A.Yoshida, Nature 216 (1967) 275.
[19] A.Yoshida, Biochem. Gen. 1 (1967) 81.