THE NATURE OF THE ALKALINE DISSOCIATION OF THE GLUTAMIC DEHYDROGENASE MOLECULE[1]

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It has been shown by Jirgensons (1961) that beef liver glutamic dehydrogenase (GDH) is split by sodium decyl sulfate (SDS) into about 20 subunits. The dissociation into such subunits by SDS, as well as by such reagents as urea and guanidine hydrochloride, has been confirmed by Wolff (1962), Frieden (1962) and Reitel.[2] Light scattering measurements, performed in this laboratory (Fisher, McGregor and Power, 1962), indicated a dissociation of GDH into units of similar average molecular weight at either very high or very low pH in the absence of such agents. We present evidence here that the dissociation of GDH into these subunits is due to the breaking of tyrosyl hydrogen bonds.

In Figure 1, the solid circles represent the reciprocal of the weight average molecular weight, $\bar{M}_w$, plotted against pH.[3] It can be seen that between pH 9.5 to 11.5 the enzyme undergoes a dissociation

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[2]Personal communication.

[3]The interaction constant, B, is negligible on this scale. It should be noted that since all of the data shown here were obtained with enzyme concentration of 1.0 mg per ml, the enzyme was already partly dissociated by the dilution effect (Olsen and Anfinsen, 1952) even at pH 7.6. $1/\bar{M}_w$ for the molecule dissociated maximally by dilution (0.1 mg/ml) and for the undissociated molecule (5 mg/ml), are shown by the upper and lower dotted lines, respectively. Since, as will be shown elsewhere, the dilution dissociation does not involve the breaking of tyrosyl hydrogen bonds, it does not affect the qualitative argument and will be disregarded here.

into about 18 subunits. This agrees well with the 18 to 25 N terminal amino groups measured by Jirgensons, for the SDS split enzyme.

The open circles in Figure 1 represent the optical density measured at λ =295, (OD_{295}), at the indicated pH's. The solid line is the theoretical titration curve for $pK_{obsd.}$=10.8. The spectrophotometric titration of the phenolate groups of GDH can be seen to follow this simple theoretical curve quite closely, except at very high pH's where phosphate dependent aggregations become noticeable. (At higher phosphate concentration both the scatter and OD curves are shifted to higher pH's with $pK_{obsd.}$=11.2. The intrinsic pK, however, is about 10.2 under those conditions.)

Other proteins have been shown to contain phenolic groups some or all of which remain undissociated unless exposed to pH's above 13 for some period of time (Crammer and Neuberger, 1943)(Tanford, Hauenstein, and Rands, 1955); such groups have been termed, "abnormal" or "buried". Clearly, none of the tyrosine residues of GDH can be considered abnormal in this sense, as their titration is essentially complete at pH 12.2. However, certain time dependencies do occur in the spectrophotometric titration at lower pH's.[4] Apparently such abnormality is not an all-or-none phenomenon, and we must recognize varying degrees of accessibility to solvent among protein phenolate groups.

It is clear that there is a close correspondence between the ionization of the phenolate hydroxyl groups and the decrease in $\bar{M}_w$ of GDH,

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[4] The experimental values obtained for both $1/\bar{M}_w$ and OD are time dependent functions. The values plotted here represent the maximum values of $1/\bar{M}_w$ (the greatest degree of dissociation) and the OD obtained at about the same time period. This maximum is obtained generally in the first 10 minutes after addition of the enzyme. This dissociation is followed by slower secondary reactions, which are time and pH dependent, and are reflected by changes in both the light scatter and optical density measurements. The downward trend in the $(1/\bar{M}_w)$ curve observed at pH 12.6 and above, is the result of a different secondary reaction whose occurrence and rate are dependent upon phosphate ion concentration. These secondary reactions will be discussed in a later communication.

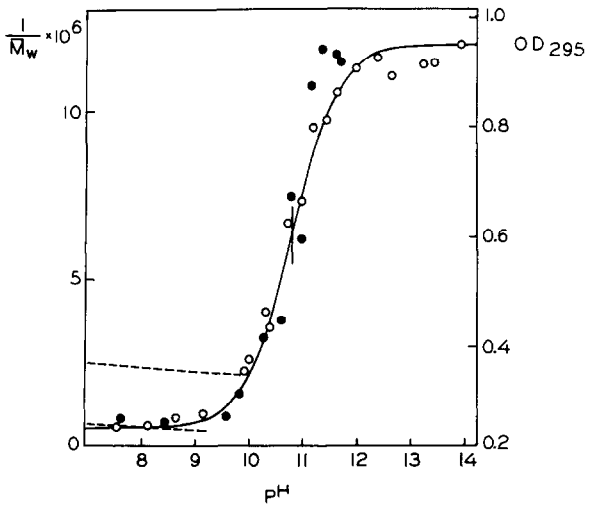

FIGURE 1. The reaction mixture contained 0.1M KCl adjusted to the proper pH with concentrated potassium hydroxide, 0.01M phosphate, 1.0 mg/ml enzyme. The enzyme was supplied by California Corporation for Biochemical Research or by Nutritional Biochemical Corporation and was dialyzed against 4 changes of 0.2M potassium phosphate pH 7.6. The enzyme solution was filtered through 0.45μ Millipore filters prior to use, all other solutions were filtered through 0.22μ Millipore filters. The temperature was 27±2° c. The enzyme was added last in each case. The pH was determined on a pHM4 Radiometer pH meter. $1/\bar{M}_w$ (●, left hand ordinate) was measured in an Aminco absolute light scattering photometer using a 10 millimeter square quartz cuvette in a specially designed adapter, at a wave length of 436mμ. $1/\bar{M}_w$ was calculated from Rayleigh ratio (R_{90}) measurements corrected for solvent scatter (Kremen and Shapiro, 1954). R_{90} measurements were corrected for reflection factor but for neither dissymmetry nor polarization. The specific refractive index was measured in a Phoenix Brice differential refractrometer and found to be 0.182.

Δ OD_{295} measurements (○, right hand ordinate) were made on identical separate solutions in a Beckman DU Spectrophotometer equipped with a photomultiplier against an identical solution at pH 7.63. Small but significant corrections for light scatter were made by the extrapolation procedure of Hamilton (1960). The slit-width was 0.2 millimeters.

The solid line represents the equation:

$$pH = pK_{obsd.} + \log \left(\frac{\Delta OD_{295}}{\Delta OD_{295\ max} - \Delta OD_{295}} \right)$$ (Hermans and Scheraga, 1961)

where Δ OD_{295} represents the difference between OD_{295} at a given pH and that at pH 7.6, the curve was drawn by adjusting the value of $pK_{obsd.}$ (vertical dash) to fit the spectrophotometric data.

and that both of these properties follow the simple theoretical curve for the titration of a weak acid.[5]

The most simple and direct explanation of this correspondence is that the individual peptide chains are held together by hydrogen bonds formed by tyrosyl donors and some yet unidentified acceptors. (This conclusion is not intended to imply either that each of the tyrosyl hydrogen bonds present is engaged in holding the peptide chains together, or that there are no other forces or bonds involved in such inter chain binding. It is quite possible that some tyrosyl residues are involved in intra chain hydrogen bonds; it is also possible that at a pH high enough to dissociate phenolic hydroxyl groups, other groups which are normally involved in inter chain bonding are now dissociated.)

However, even with the limiting considerations given above, it is clear that certain tyrosyl hydrogen bonds are both necessary and sufficient to hold the individual peptide chains of GDH together in higher molecular weight aggregates, and that the rupture of such bonds in the native enzyme is the cause of the alkaline induced dissociation of the protein molecule.

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[5] While $1/\bar{M}_w$ is not the most appropriate function to compare with a titration curve, expressing the data in terms of degree of dissociation would require assumptions as to the number and size of the intermediate states of the dissociation reaction.

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