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# Dissociation of Glyceraldehyde-3-Phosphate Dehydrogenase with Sodium Dodecyl Sulfate[1]

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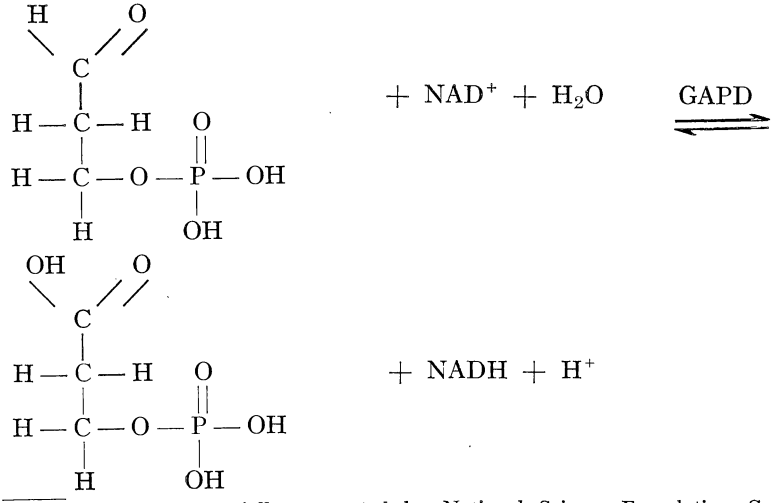
*Abstract.* Rabbit muscle glyceraldehyle-3-phosphate dehydrogenase (GAPD) was dissociated into a subunit species via lauryl sulfate (LS). The incubation mixture prepared from active GAPD $10^{-5}$ M and LS $10^{-3}$ M showed a rapid loss of initial activity. Within 30 minutes after the addition of the powdered LS, there was a 50 percent loss in initial enzymatic activity. The appearance of the subunit species was verified by Sephadex (a crossed-linked dextran) molecular sieve studies. Using a column buffer containing 0.01 M acetic acid, 0.10 M NaCl and 0.005 M LS at 25° C., with a pH of 6.1 ± 0.1, elution patterns of GAPD, conalbumin, hemoglobin and lysozyme were used to calibrate a G-100 Sephadex column. From the Sephadex gel filtration studies, it was discerned that GAPD in the presence of LS acts as a dimer.

A study of the reaction of glyceraldehyde-3-phosphate dehydrogenase (GAPD) and lauryl sulfate (LS) was begun in the summer of 1967 with a grant from the National Science Foundation. The purpose of the study was to attempt the dissociation of the native enzyme with LS and to determine the molecular weight of the newly formed subunit species. As a result of this work, such phenomena as active subunits, subunit interactions, polymeric hydrophobic binding, reactivation of the subunits, and differences in chemical composition could now be investigated.

GAPD is a key enzyme in the glycolytic conversion of glucose to pyruvic acid, and as such has an important role in the carbohydrate metabolism of most organisms (1). Its catalytic action is expressed in the following reaction:

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The accepted molecular weight for GAPD reported as late as 1965 is 144,000 (1). Harris and co-workers in Cambridge, England, also report that the native enzyme is a tetramer which is composed of four identical protein chains (1). Little is known about the chemical binding of the monomers.

In this country, Spiros M. Constantinides and W. C. Deal of Michigan State University have reported in recent communications the reversible dissociation of the polymeric enzyme into two types of "subpolymer species" (2, 3). The studies involved low temperatures and dilute enzyme concentrations, and identification of subpolymer species by sucrose gradient centrifugation.

## Experimental Procedure and Results

### *Preparation of GAPD-LS Incubation*

Active GAPD-$NH_4SO_4$ suspension as supplied by Sigma Chemical Co., St. Louis, Missouri, was dialyzed for 24 hours against 0.10 M tris with a pH of 7.5 at a constant temperature of 4° C. It was hoped that in the process of dialysis any extramolecular particles and the salt would be removed from the enzyme. From the dialyzed enzyme solution, a mixture containing $6.5 \times 10^{-5}$ M GAPD and $7.5 \times 10^{-3}$ M LS was prepared, giving an approximate molar ratio of 1 : 100. The pH of the solution was 7.1 $\pm$ 0.1. The LS was added as a dry powder and the reaction was said to be initiated upon the addition of the LS.

### *Preliminary Analysis of GAPD-LS Incubation*

A typical elution pattern of active GAPD through the G-75 Sephadex column shows the enzyme being eluted in the fifth or six fraction (Figure 1). Activity determinations on the eluted material showed that the enzyme activity was not only maintained, but increased slightly over the control.

From the GAPD-LS reaction mixture, 0.50 ml. aliquot was placed upon a G-75 column. A typical elution pattern for this series of separations can be found in Figure 1. A series of four separations showed significant absorption in the 13th through the 18th fractions at 280 millimicrons. Activities were carried out on the absorbing materials. The aforementioned fractions showed no enzymatic activities. An activity study was carried out on the GAPD-LS incubation. The systems were prepared:

1. A solution of $6.5 \times 10^{-5}$ M GAPD plus $7.5 \times 10^{-3}$ M LS.
2. A solution of $6.5 \times 10^{-5}$ M GAPD.

In the first system the molar concentration of LS is 100 times in excess to the GAPD. The second system served as a control and the activity of each system was recorded as a function of time. The results are contained in Table 1.

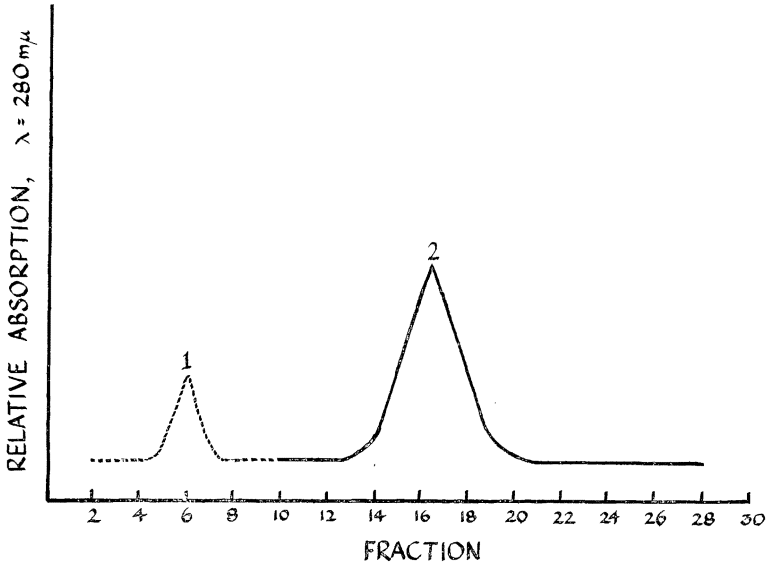


Figure 1. Dashed line is the normal elution peak for native GAPD on a G-75 column. The solid line is the displaced peak for the GAPD-LS incubation mixture.

**Table 1**

Activity of GAPD-LS Incubation

| Time | Activity-Control GAPD | Activity-Reaction GAPD-LS |
|---|---|---|
| Initial | 100% active | 100 % active |
| 15 minutes | 100% active | 60 % active |
| 30 minutes | 100% active | 0.5% active |
| 45 minutes | 100% active | 0.2% active |
| 12 hours | 100% active | No activity |
| 24 hours | 100% active | No activity |

With this data it seemed feasible to submit the idea that the disappearance of the native enzyme peak in the elution pattern and the simultaneous appearance of a lower weight particle correlated well with the loss of enzymatic activity. It is also indicative of a change in the quaternary or tertiary structure of the protein. However, with subsequent column separations, recipitation of the GAPD-LS mixture on the column occurred. It was believed that when placed on the column, the GAPD-LS mixture, in its incumbation environment, was disturbed. The presence of LS in the column buffer facilitated GAPD-LS separations.

*Molecular Weight Determination of the LS Treated GAPD.*

*Phase I: G-75 sephadex column.* Specifications state that the upper exclusion limit of a G-75 column is 50,000. This porosity of sephadex resin was chosen to test the possibility that GAPD dissociates in the presence of LS into four monomers of molecular weight approximately 36,000 (1). Hemoglobin, lysozyme and the GAPD-LS mixture were used in this series of separations.

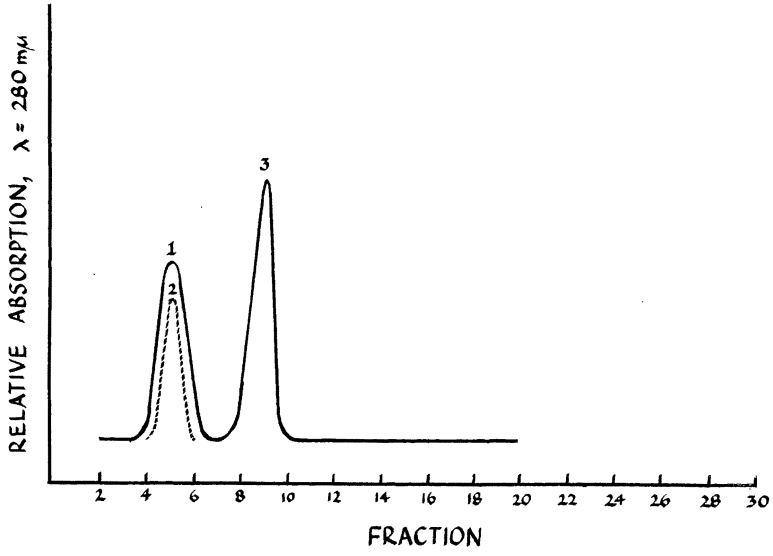


Figure 2. Elution pattern for hemoglobin, GAPD-LS and lysozyme on G-75 Sephadex column. Peak 1 is hemoglobin; peak 2, GAPD-LS; peak 3, lysozyme. See Figure 3 for protein concentration.

Figure 2 is representative of these separations. One would expect hemoglobin with a molecular weight of 68,000 to be eluted in one of the early fractions because its molecular weight is greater than the upper exclusion limit of G-75 resin, and lysozyme to be eluted later.

Hemoglobin was eluted as expected. However, lysozyme was eluted within one fraction of hemoglobin. After lysozyme was placed on the column, a white precipitate formed which disappeared shortly. Possibly, the formation of precipitate led to a polymer of lysozyme thereby increasing its molecular weight. The eluted material of the GAPD-LS mixture appeared in the same fraction as did the hemoglobin, indicating a similar molecular weight.

*Phase II: G-100 sephadex column.* To determine the upper molecular weight limit of the GAPD-LS subunit species, a fractionation range of 100,000 to 5,000 was obtained with sephadex G-100 resin. It was assumed that the GAPD-LS subunit would enter the molecular sieve if it were less than 100,000. Also since it appeared that the subunit species and hemoglobin have similar molecular weights as was seen in the G-75 study, one could expect to find them eluted in the same fraction.

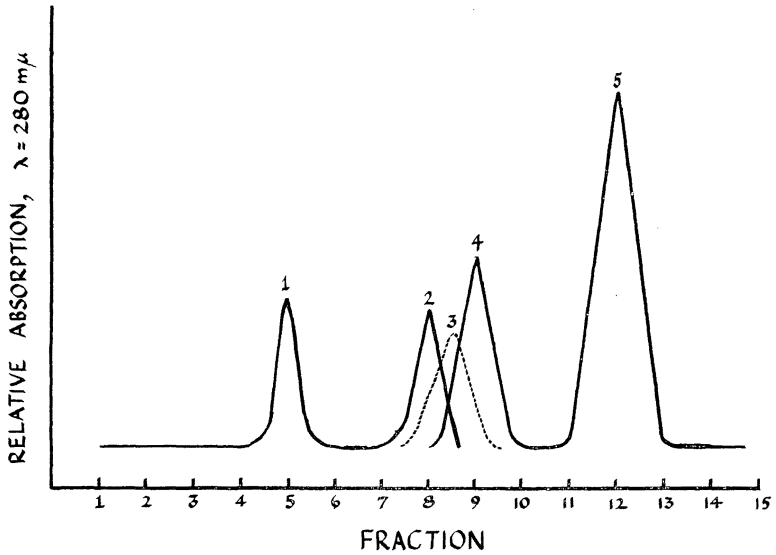


Figure 3. Elution pattern for Sephadex G-100 column. Peak 1 is native GAPD; peak 2, conalbumin; peak 3, GAPD-LS; peak 4, hemoglobin; peak 5, lysozyme. The preparation of each protein was such that a 0.25 ml. aliquot of protein solution contained 1.00 mg. of protein; the solutions were prepared in cation-free distilled water.

In this G-100 series, GAPD, conalbumin, GAPD-LS, hemoglobin, and lysozyme were used to calibrate the column. Figure 3 is a representative composite of these G-100 separations. As can be seen, lysozyme comes off in the twelfth fraction; seven separations were used to determine this value. As stated before, lysozyme does precipitate when placed upon the column.

The elution volume of GAPD was previously determined on a non-LS-G-100 sephadex column. When a sample of GAPD was placed on a column with buffer containing LS, the fraction with maximum absorption was eight instead of five. This can be explained quite easily.

The buffer contained an excess of LS five times that of the incubation mixture and thus one could expect dissociation to occur on the column. Table 2 contains a summary of the G-100 separations.

Table 2

Summary of G-100 Separations

| Protein | Molecular Weight | Fraction | Elution Volume |
|---|---|---|---|
| GAPD | 144,000 | 5 ± 1 | 10.0 ± 1 ml. |
| GAPD-LS | | 8.5 ± 0.5 | 17.0 ± 1 ml. |
| Conalbumin | 76,000 | 8.0 ± 0.5 | 16.0 ± 1 ml. |
| Hemoglobin | 68,000 | 9.0 ± 0.5 | 18.0 ± 1 ml. |
| Lysozyme | 14,000 | 12.0 ± 0.5 | 24.0 ± 1 ml. |

## Discussion

It appears that the GAPD-LS subunit particle has a molecular weight between 68,000 and 76,000. Accepting the molecular weight of native GAPD to be 144,000, it would seem that this polymeric enzyme is dissociating into a subpolymer with a molecular weight of 72,000, which is one-half the molecular weight of the dimer GAPD. At first, the conclusion that GAPD is a dimer seems to be contrary to the work of Harris (1) and in partial support of Constantinides and Deal (2, 3). However, the authors wish to point out that GAPD appears to exist as a dimer in the presence of LS. Under other experimental conditions, such as those reported by Constantinides and Deal, the native enzyme apparently dissociates into four monomers.

### References

1. Harris, J. I., and R. N. Perham. 1965. Journal of Molecular Biology, Vol. 13, pp. 876-884.
2. Constantinides, Spiros M., and William C. Deal. Abstracts of the American Chemical Society Meeting, September, 1967.
3. Constantinides, Spiros M., and William C. Deal. Abstracts of the Federation Proceedings, 52nd Annual Meeting, April, 1968.

 