MEMBRANE-ENZYME BINDING PHENOMENA AND MUSCLE GLYCOLYSIS*

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The great bulk of the work in studies of post-mortem muscle has involved the structural proteins or the connective tissue proteins, although it is well recognized that many of the changes brought about in these proteins are influenced significantly by the soluble enzymes of the muscle cell. We have studied how the activities of some of the glycolytic enzymes in chicken muscle are affected by interaction with subcellular particulate structures with a view to understanding what controls the functioning of these enzymes post-mortem.

I shall discuss two classes of enzymes which we have studied. One of these classes is exemplified by the dehydrogenases, lactate dehydrogenase (LDH) and glyceraldehyde-phosphate dehydrogenase (GAPDH). These fall under the category of enzymes which are very easily solubilized and have, in fact, been classically considered to exist as soluble entities in the sarcoplasm. The second class are those requiring more vigorous treatment to remove them from the particulate structures and have been recognized as existing at least in part in association with the particulate structures of the cell. The proposals that I am putting forward involve reversible binding and solubilization of the enzymes. Thus, these enzymes are not intrinsic parts of membranes nor do they remain in the particulate phase at all times. These enzymes are representative of the "extrinsic" or "peripheral" proteins of membranes described in the paper by Dr. M. Stromer.

In many cases, the binding-solubilization which takes place may involve structures other than membranes, for example, Pette's group in Germany has shown that a number of the glycolytic enzymes can interact with purified actin (Arnold and Pette, 1968, 1970; Arnold et al., 1971), and Amberson and his coworkers at the Marine Biology Laboratory at Wood's Hole, Massachusetts, have demonstrated by zonal electrophoretic techniques that the soluble proteins of the muscle cell can bind to myosin and other structural proteins under the proper conditions (Amberson and Bauer, 1971).

My suggestions are going to be tentative. They are meant more to stimulate discussion and serve as a basis for further experimentation than as a final pronouncement on the exact role of the interaction between enzymes and the particulate structures of muscle.

To consider that reversible binding-solubilization plays a role in muscle metabolism, solubilization of an enzyme must be demonstrated to be brought about by conditions which are reasonably physiological whether it be a change in pH, ionic strength, or the concentration of a metabolite. It is worthwhile to note that all of the enzymes which will be discussed can be specifically solubilized by either a substrate, product, or an effector of the particular reaction sequence in which the enzyme is involved.

The first enzyme which we examined and the one with which we have done the most work is lactate dehydrogenase. We chose this enzyme because earlier studies had indicated that it was approximately an average enzyme in its degree of binding to the membrane of the erythrocyte (Green et al., 1965). Lactate dehydrogenase is a tetrameric enzyme composed of two different subunits giving five isoenzymic forms which are labeled as isoenzymes 1, 2, 3, 4, and 5 depending on their particular subunit makeup. LDH1 contains all heart-type or H subunits and lactate dehydrogenase 5 contains four M or muscle-type subunits. Lactate dehydrogenase reoxidizes NADH using pyruvate as the electron acceptor, producing lactate and regenerating the oxidized form of the cofactor, NAD+, for utilization by glyceraldehyde-3-phosphate dehydrogenase. The latter enzyme is very sensitive to inhibition by NADH.

Lactate dehydrogenase of chicken breast muscle is one of the easily solubilized enzymes that I referred to above. It is very sensitive to solubilization by increasing pH as shown in Figure 1 where an inflection point is observed around 7.2. This is in the physiological range. It is also very sensitive to solubilization by ionic strength as shown in Figure 2. The ionic strength required for solubilization is much less than what is usually considered physiological. I do not intend in this paper to get into a detailed discussion of hypotheses on the location and form of the ions in the muscle cell. Suffice it to say that there is one school of thought that considers most if not all of the ions in the muscle cell to be complexed to proteins (Ling, 1962, 1969; Cope, 1967). It is perhaps more to the point that under conditions which approximately duplicate those in situ, it can be demonstrated that the enzyme is capable of binding to the ultrastructure of the muscle cell (Ross, 1973). This work is illustrated by data shown in Table 1. The lactate dehydrogenase activity is expressed on the basis of the amount of water in either the muscle or the press juice. Chicken breast muscle was first centrifuged to remove the press juice, and the activities obtained are given in the table. The pressed muscle was then imbibed in a pH 7.5 buffer of ionic strength 0.2, which should be more than enough to solubilize the enzyme according to our in vitro studies. A small amount of the enzyme leaches out during this process but in any case, the activity on a per ml water basis in the imbibed muscle goes down since it is taking up buffer with no enzyme in it. The activity in the imbibed muscle is also lower than that in the first press juice. This imbibed muscle now is soaked for various time periods in the original press juice. Assuming that the muscle segments are freely permeable to the lactate dehydrogenase in the press juice, an equilibrium
Figure 1: Substitution of chicken breast muscle pH as a function of pH (Hutton and Westport, 1966).
Fig. 2. Solubilization of chicken breast muscle LDH as a function of ionic strength (Hultin and Westort, 1966) ○ - NaCl; ● - CaCl₂; △ - MgCl₂.
TABLE 1. REINTRODUCTION OF LDH INTO PRESSED CHICKEN BREAST MUSCLE

<table>
<thead>
<tr>
<th>Sample</th>
<th>Activity (umoles/min) per ml water in Muscle</th>
<th>Press juice</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole muscle</td>
<td>1745</td>
<td></td>
</tr>
<tr>
<td>Pressed muscle</td>
<td>2517</td>
<td>1184</td>
</tr>
<tr>
<td>Imbibed muscle</td>
<td>793</td>
<td></td>
</tr>
<tr>
<td>Restored muscle</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5 hr</td>
<td>1570</td>
<td>900</td>
</tr>
<tr>
<td>24 hr</td>
<td>1945</td>
<td>587</td>
</tr>
</tbody>
</table>

The phosphate buffer used for imbibition was at pH 7.5 and an ionic strength of 0.2. The experimentally determined ionic strength of the press juice was approximately the same as the buffer.

should be reached between the muscle and the press juice. That is, on a per ml of water basis, the activities in both the muscle and the press juice should be the same. This, in fact, does not happen, but the muscle takes up much more enzyme than would be predicted based on an equilibrium distribution. We interpret this excess enzyme taken up to be due to binding of the enzyme to the ultrastructure of the muscle. This happens even though the ionic strength and pH were such that the enzyme would have been solubilized in vitro. Thus, under conditions similar to those encountered in situ, chicken breast muscle LDH can apparently bind to the subcellular particulate structures.

Figure 3 illustrates the observation that lactate dehydrogenase from chicken breast muscle, which is approximately 98% or more LDH5, is solubilized by its cofactor, NADH. It is also important to notice that the effectiveness of NADH as a solubilizing agent is highly dependent on the amount of the insoluble muscle protein which is present in the suspension. That is, its effectiveness decreases as the amount of this insoluble protein increases.

Several other metabolites have been examined for their ability to solubize LDH, but none of them were as effective as shown in Table 2 with the exception of NADPH (Melnick and Hultin, 1970). The oxidized form of the cofactor, NAD+, was a less efficient solubilizing agent of the enzyme.
Fig. 3. Solubilization of LDH from the particulate fraction of homogenized chicken breast muscle as a function of NADH concentration at various levels of tissue particulates in 10 mM imidazole buffer at pH 7.0. The tissue concentrations are equivalent to those corresponding to (w/v) homogenates. A 1% preparation contains approximately 2 mg of insoluble protein per ml.
TABLE 2. SOLUBILIZATION OF LDH BY CELL METABOLITES STRUCTURALLY RELATED TO NADH

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>% LDH in supernatant</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>5.5</td>
</tr>
<tr>
<td>NADH</td>
<td>87.7</td>
</tr>
<tr>
<td>NAD⁺</td>
<td>39.4</td>
</tr>
<tr>
<td>NADPH</td>
<td>90.7</td>
</tr>
<tr>
<td>NADP⁺</td>
<td>32.2</td>
</tr>
<tr>
<td>ATP</td>
<td>14.9</td>
</tr>
<tr>
<td>ATP + Mg²⁺</td>
<td>23.2</td>
</tr>
<tr>
<td>Adenosine</td>
<td>6.2</td>
</tr>
<tr>
<td>Nicotinamide</td>
<td>6.8</td>
</tr>
<tr>
<td>Pyrophosphate</td>
<td>22.8</td>
</tr>
</tbody>
</table>

1 The concentration of all metabolites used was 0.4 mM. The pH was 6.7, obtained with 10 mM imidazole buffer.

Lactate dehydrogenase 3, which is the hybrid form with 2 M subunits and 2 H subunits binds to the particulate structures of muscle. It is solubilized by the same factors as solubilize lactate dehydrogenase 5, that is pH, ionic strength, and NADH. It is, however, solubilized at lower pH's, lower ionic strengths, and is particularly susceptible to solubilization by NADH (Nitisewojo, 1972).

Lactate dehydrogenase 1, which is composed of the four H subunits does not bind to the particulate fraction of either skeletal muscle or heart muscle under any of the conditions that we have examined so far. It is assumed that this is due to the fact that the charge distribution on the enzyme is similar to that on the particulate structures so that they are not oppositely charged and do not interact. It is probable that the muscle-type subunit is responsible for the interaction of the enzyme with the cell particulate structures.

Up to this point, we had worked on the hypothesis that "soluble" enzymes like LDH were permanently associated with the ultrastructure of the cell. Based on the binding data, it became more reasonable to expect that there was a reversible binding-solubilization between the
enzyme and the particulate structures which could be controlled by environmental factors. The next step, therefore, was to determine what kinetic differences there were between the enzyme when bound and when in the soluble phase and to determine if there was any logical reasons for differences that might be observed.

To do this, we studied chicken breast muscle lactate dehydrogenase bound and soluble. We used a stopped-flow procedure (Ehmann and Hultin, 1973a) so that we could keep the enzyme concentration reasonably high to duplicate the physiological conditions. We also employed a sensitive end-on-photomultiplier tube so that we could use fairly high levels of insoluble structural protein which, as shown above, was necessary to maintain the enzyme in the bound state in the presence of the cofactor, NADH. We carried out these studies over a range of temperatures from 40 to 4°C to represent roughly the range from cold storage of the muscle to that of the physiological temperature.

In Fig. 4 are shown the Arrhenius plots of soluble and bound lactate dehydrogenase at equal enzyme concentrations. The effect of temperature on the enzyme in both phases was similar, but the bound enzyme at equal concentrations was less active by a factor of approximately 3 or 4 than the soluble enzyme.

Fig. 5 shows a plot of $K_M$ versus temperature for bound and soluble LDH. There was a small but consistent increase of $K_M$ for the bound enzyme at all temperatures compared to the soluble. This indicates that the soluble enzyme has slightly more affinity for substrate than does the bound. There is a significant change in $K_M$ of both bound and soluble LDH with temperature.

Inhibition of LDH can occur through the formation of an abortive ternary complex of enzyme, NAD$^+$ and pyruvate (Zewe and Fromm, 1962), and the formation of this abortive ternary complex has been suggested as a control mechanism for the regulation of LDH activity in situ (Kaplan et al., 1968). We studied the effect of binding on the formation of the abortive ternary complex by a stopped-flow procedure in which the NAD$^+$ was incubated with the enzyme prior to the assay. We did this since in the cell the ratio of NAD$^+$ to NADH is high (Long, 1961); thus, pre-incubation should be more physiological than NAD$^+$ generation during the reaction. We adjusted the incubation time of the enzyme with NAD$^+$ such that maximal inhibition was attained. The time required varied as a function of temperature, taking longer for the reaction to be completed at the lower temperatures (Ehmann and Hultin, 1973b). The results of these studies are shown in Fig. 6, which plots the percentage activity of the enzyme in the presence of NAD$^+$ to that in its absence versus pyruvate concentration. Again we did this at temperatures of 4, 16, 23 and 40°C, using chicken breast muscle LDH, that is, LDH5. The amount of inhibition with the soluble enzyme was much greater than that of the bound enzyme and there was little effect of temperature. In the case of bound LDH5, however, there was a difference depending on the temperature. At 40°C, which is close to
Fig. 5. Relationship between the $K_M$ of soluble and bound chicken breast muscle LDH and temperature.
Fig. 6. Percentage of initial velocity of lactate dehydrogenase in the presence of NAD$^+$ compared to that in the absence of NAD$^+$ as a function of [KPyr] $\times 10^4$ M at different temperatures.
the physiological temperature of the chicken, there was essentially no inhibition. There was a significant amount of inhibition at the lower temperatures, but it was much less than was observed with the soluble enzyme. It is apparent that the binding of LDH from chicken breast muscle to the particulate fraction does give it some protection from inhibition by pyruvate and NAD⁺.

We carried out similar studies on the protection of lactate dehydrogenase from inhibition by pyruvate and NAD⁺ on binding. There was no evidence of any protection in the case of this isozyme (Nitisewojo, 1972). Obviously, the presence of the two heart-type subunits in LDH3 has a profound effect on the ability of the enzyme to interact with NAD⁺ and pyruvate.

Fig. 7 gives a summary of our hypothesis for the control mechanism of lactate dehydrogenase based on the kinetic and binding data which we have obtained to date (Hultin et al., 1972). Glycogen or glucose is the energy source of muscle via glycolysis. Oxidation of glyceraldehyde-3-phosphate to 1,3-diphosphoglycerate by glyceraldehyde-3-phosphate dehydrogenase requires NAD⁺ and produces NADH. The latter must be removed since it is a very potent inhibitor of glyceraldehyde-phosphate dehydrogenase. There is competition between the electron transport chain of the mitochondria and LDH for the electrons of NADH. The former is more efficient and should be the pathway of choice under most conditions. It is that way ordinarily because of low NADH levels in resting muscle. LDH is bound and relatively inactive. When oxygen is depleted, as in stress, or in postmortem muscle, mitochondrial oxidations cease. The NADH level increases, solubilizes the LDH which can then reoxidize the NADH more efficiently than the bound LDH. This mechanism allows the maintenance of glycolysis under anaerobic conditions while at the same time minimizing competition for electrons between LDH and mitochondria under aerobic conditions. The data on inhibition by NAD⁺ and pyruvate would indicate that although the soluble enzyme is potentially more active, it is also more subject to control. On the other hand, the bound enzyme, although inherently less active, can function even in the presence of high levels of NAD⁺ as would be expected to be present in resting muscle.

Fig. 8 indicates an added factor, which is that under any particular set of conditions of substrate concentration, pH, etc., a whole range of activities of LDH is possible depending on the amount of enzyme that is bound as is illustrated in this figure by the arrow. Thus the relative activity of lactate dehydrogenase could be controlled by factors other than the metabolites taking part in the reaction. An example of this would be a change in the ionic environment of the cell in the area where the enzyme is functioning.

We examined next the glyceraldehyde-3-phosphate dehydrogenase (GAPDH) enzyme from chicken breast muscle. We chose this one for several reasons. First of all it has many similarities to LDH. Besides being a dehydrogenase, it is composed of four subunits and has approximately the same molecular weight. In addition, it is functionally
Fig. 7. Proposed scheme for the role of reversible binding-solubilization of lactate dehydrogenase in carbohydrate metabolism.
Fig. 8. Initial velocity of lactate dehydrogenase as a function of pyruvate concentration in the soluble (○) and bound (●) phase. The arrow represents the potential range of activity of the enzyme at that particular pyruvate concentration dependent on the percentage of enzyme that is in the bound phase.
related to lactate dehydrogenase; the two work in close rapport with LDH serving to regenerate cofactor for glyceraldehyde-3-phosphate dehydrogenase. Like LDH, glyceraldehyde-3-phosphate dehydrogenase was shown to be a "loosely" bound enzyme of the muscle particulate fraction (Dagher, 1971). It was solubilized somewhat more easily than the LDH. The pH solubilization curve was very similar to that of LDH except the inflection point of approximately 7.0 was just slightly lower than that of LDH. NADH did not solubilize the enzyme at any level studied while the reduced coenzyme, NADH, only worked at a very high non-physiological concentrations. On the other hand, both the substrate, glyceraldehyde-3-phosphate, and 2,3-diphosphoglycerate had specific solubilizing effects on the enzyme.

We again examined some kinetic characteristics of the enzyme when bound and when in the soluble phase. Results of initial velocity vs. (S) are shown in Fig. 9. The soluble enzyme shows substrate inhibition while the bound does not. A summary of some of the kinetic constants of GAPDH is shown in Table 3. The \( V_{\text{max}} \) value is decreased on immobilization as for LDH but the \( K_M \) value is decreased as well. The inhibitor constants show that soluble enzyme is considerably more subject to inhibition by NADH than the bound. We also have evidence that a portion of the glyceraldehyde-3-phosphate dehydrogenase of chicken breast muscle is bound \textit{in situ} (Melnick and Hultin, 1973).

We suggest that modification of the kinetic properties of GAPDH by solubilization or binding may be important in the regulatory mechanisms of the muscle cell. The lower \( K_M \) value of the bound enzyme for glyceraldehyde-3-phosphate compared to the soluble form enables the bound enzyme to operate close to its minimal performance at low substrate concentrations. In resting muscle, low glycolytic activity prevails but in working muscle there is an increase in the glycolytic rate. This increase in the rate of glycolysis leads to higher concentrations of many of the glycolytic intermediates including glyceraldehyde-3-phosphate. If these compounds are involved in solubilization of glyceraldehyde-3-phosphate dehydrogenase \textit{in situ}, the enzyme would be solubilized. The enzyme in soluble form has a higher maximal velocity and seems better suited to function in such a situation, that is, that of high substrate concentration. The soluble enzyme is also more sensitive to inhibition by NADH; thus the cell would have better control of the activity of this enzyme under conditions where its maximal activity is high.

Arnold and Pette (1970) have shown that fructose diphosphate, dihydroxyacetone phosphate, ATP, ADP and Pi inhibit the absorption of aldolase to actin within the physiological range of concentrations of these components. They have also shown that binding of aldolase to F-actin doubles the \( V_{\text{max}} \) and increases the \( K_M \) for fructose diphosphate by almost one order of magnitude. Aldolase appears to be an easily solubilized enzyme, being completely removed by moderate concentrations of salt.
Fig. 9. Initial velocities of soluble and bound glyceraldehyde-3-phosphate dehydrogenase as a function of glyceraldehyde-3-phosphate concentration. The concentration of enzyme was 5 μM in all cases (Dagher and Hultin, 1973).
Hexokinase and phosphofructokinase represent the class of enzymes requiring more vigorous conditions to solubilize than the ones previously mentioned. Hexokinase is the rare glycolytic enzyme that has been considered to exist at least partly bound to subcellular structures. This has been due to two factors, one is the aforementioned fact that it is not easily solubilized, and second, it is associated with mitochondria, and mitochondria have been studied from many tissues in some depth.

For this reason, hexokinase, in both the soluble and bound form, has been studied to quite some extent. The kinetic parameters of $K_M$ for glucose and ATP, and also $V_{\text{MAX}}$ comparisons between bound and soluble enzymes, have varied a great deal depending on the source of the enzyme. It has been demonstrated repeatedly however, that the soluble enzyme is much more sensitive to inhibition by glucose-6-phosphate than is the bound enzyme.

To solubilize hexokinase, relatively vigorous conditions are required. Combinations of reasonably drastic pH's, high salt concentrations, (~0.8M) and detergents (often with sonication), will give good solubilization (Southard and Hultin, 1972a). Hexokinase can also be solubilized by low levels of glucose-6-phosphate and considerably higher levels of ATP (Rose and Warms, 1967), the latter being a substrate of the reaction and the former a product.

We found about 60% of the hexokinase of chicken breast muscle to be associated with the particulate fraction. To study the effect of solubilization, however, we purified mitochondria and worked strictly with the mitochondrial enzyme (Southard and Hultin, 1972b). We showed that the mitochondrial enzyme was essentially all one isoenzymic form, the so-called type II. We solubilized mitochondrial hexokinase by using potassium chloride at a final concentration of 0.8M and citrate to a final concentration of 10 mM with pH adjusted to 5.5. We avoided the use of detergents since these sometimes have strange effects on some
enzymes. This suspension then was treated with ultrasonic energy and then separated by centrifugation. The procedure was repeated which gave solubilization of greater than 90% of the total bound hexokinase. After separation of the soluble enzyme, the supernatant fraction must be quickly adjusted to pH 7 in order to maintain activity.

Atkinson (1968) has proposed the concept of energy charge which is defined in equation (1) and is related to the amount of high energy phosphate bond compared to the total nucleotide.

\[
\text{Energy charge} = \frac{(\text{ATP}) + \frac{1}{2} \text{ (ADP)}}{(\text{ATP}) + \text{ (ADP)} + \text{ (AMP)}} \quad \text{Eq. (1)}
\]

He has found that many control enzymes are sensitive to this factor. The response of the enzyme is usually such that the activity of an enzyme involved with energy generation decreases with increased energy charge and the activity of one involved with energy utilization increases with increased energy charge. In the living muscle cell, the energy charge varies over the range from about 0.8 to 0.9, although this will drop postmortem. The response of bound and solubilized mitochondrial hexokinase from chicken breast muscle to energy charge which we have determined as shown in Fig. 10. Over the physiological range, bound hexokinase is much more sensitive than is the soluble although the difference decreases at the low energy charges which one would see with time postmortem. We have shown that this difference in response to energy charge is a reflection of a significantly greater inhibition of the bound enzyme by ADP than the soluble (Southard and Hultin, 1972b).

The general shape of the hexokinase response curve to energy charge indicates that it takes part principally in an energy-utilizing pathway. This means that the reaction catalyzed by the enzyme contributes to a biosynthetic pathway, that is, one requiring a net expenditure of energy. Thus, the glucose-6-phosphate produced by the hexokinase-catalyzed reaction in muscle should be principally utilized for glycogen synthesis rather than glycolysis. When glycogen is synthesized, significant levels of glucose-6-phosphate must be present since this compound activates the glycogen synthesis system. As discussed above, the soluble enzyme is more sensitive to glucose-6-phosphate inhibition than is the bound. Therefore, the bound enzyme would be better suited for producing glucose-6-phosphate for glycogen synthesis than the soluble. The association of hexokinase with the mitochondrion may occur in order to efficiently supply ATP to the enzyme for glycogen synthesis. A recent study has shown, in fact, that mitochondrial hexokinase can utilize ATP produced by mitochondria more efficiently than exogenously added ATP (Gots et al., 1972).

When glycogen stores are complete, there is no need to manufacture more. If the energy charge were high, the enzyme would be active and waste ATP. It would also produce glucose-6-phosphate, but this is a relatively poor inhibitor of bound hexokinase. A possible explanation for the control of hexokinase under these conditions would be the
Fig. 10. Effect of energy charge on hexokinase activity of the mitochondrial-bound and solubilized enzyme.
sensitivity of bound hexokinase to energy charge. As ATP is utilized and the energy charge drops, the bound enzyme is inhibited. This would provide the control to maintain energy stores without at the same time wasting energy. This suggests that perhaps soluble hexokinase takes part principally in glycolysis and is regulated by glucose-6-phosphate while the bound enzyme may be involved in glycogen synthesis and is controlled by the energy charge. The particular location of this enzyme in the cell would affect not only its kinetic properties but also the metabolic sequence in which it is active. This could have profound significance for the rate of glycolytic activity in muscle postmortem.

Many consider phosphofructokinase to be the prime point of regulation of glycolytic activity in muscle. We have found that essentially all of the PFK from chicken breast muscle is isolated with the sedimentible fraction after homogenization (Chism, 1973). Like hexokinase, it requires rather drastic treatments to solubilize it. It is extremely sensitive to inactivation by many handling procedures and, thus, the evaluation of its role in soluble and bound forms is extremely difficult. High phosphate helps to maintain stability. The pH-solubility graph of PFK is shown in Fig. 11. It is almost a completely cooperative response. Phosphofructokinase was also solubilized by one of its substrates and effectors, ATP. The concentration dependence of this is shown in Fig. 12. This solubilization by ATP is time-dependent with a half-time of approximately 20-30 sec. Phosphofructokinase is also solubilized in a time-dependent manner by ADP, but this takes well over half an hour for 50% solubilization, and we do not feel that this is a physiologically significant phenomenon.

Kinetic studies of phosphofructokinase in the bound and soluble form in any detail such that values of \(K_M\) and \(V_{MAX}\) could be calculated, have not been possible because of the difficulties of dealing with this enzyme. However, we have shown that the bound form is more active than the soluble. We have also shown that this enzyme can rebind to the particulate fraction. The conditions which lead to solubilization, i.e., high pH and high (ATP), are those which would be found in the resting cell. The enzyme would be soluble and less active. On depletion of ATP, and dropping of the pH, such as would occur postmortem or under other types of stress such as exercise, the enzyme would be rebound and would be more active and presumably would increase the rate of glycolytic activity under these conditions relative to the soluble form of the enzyme. Again, our proposal is that reversible binding-solubilization results in control of the activity of the enzyme.

In summary, we see some basic similarities in many glycolytic enzymes (and probably other soluble enzymes as well) regarding the effect of reversible interactions with cellular particulate structures. These are:

1. The substrates, products, or effectors of the reactions are capable of solubilizing the enzymes.

2. There is a reversible binding-solubilization under conditions which are reasonably physiological.
Fig. 11. Solubilization of phosphofructokinase from the particulate fraction of homogenized chicken breast muscle as a function of pH.
Fig. 12. Solubilization of phosphofructokinase from the particulate fraction of homogenized chicken breast muscle as a function of ATP concentration.
3. There are very often different properties in the unbound and bound forms. I should note here, however, that these types of differences are not mandatory for there to be an effectiveness of binding. For example, if lactate dehydrogenase and glyceraldehyde-3-phosphate dehydrogenase were bound to the same surface, just the very fact of their being closely aligned and able to pass substrate from one to the other quickly and easily, might be sufficient justification for their binding without any modification of the properties of the individual enzymes.

4. The enzymes involved are subunit enzymes which, at least under some conditions, are allosteric control enzymes, and

5. There is difficulty in studying these effects under reasonably in vivo conditions, and very often under non in vivo conditions these differences in properties are not observed. I am sure that this is one reason why more instances of these occurrences have not been reported.

Along with these basic similarities, there may also be important differences between enzymes. There may be different specific changes in the kinetic parameters of individual enzymes. It is also likely that one will see differences depending on the particular surface to which the enzyme binds. There is no reason to assume, for example, that the same properties are going to be observed when LDH binds to mitochondria as when it binds to the nucleus or to actin.

The rates of solubilization may also vary. We have done some work which indicates that the solubilization of LDH is extremely fast, faster than we can measure with a stopped-flow apparatus so that it must be at least in the milli-second range. On the other hand, both hexokinase and phosphofructokinase have a relatively slow solubilization rate with half-times of the order of half a minute. The rate of solubilization or binding may itself play a role in the control of the enzyme (Gumma and McLean, 1969).

Another important consideration in the effects of the changes in enzyme characteristics on binding in meat is the specific effect that temperature has. With lactate dehydrogenase, the effect of temperature is extremely complex. The difference isoenzymes behave differently, and the bound and soluble enzymes respond in different ways to changes in temperatures (Ehmann and Hultin, 1973a,b,c; Nitisewojo, 1972).

I have presented in this paper some data obtained in our laboratory concerning interactions of various glycolytic enzymes and cellular particulate structures as well as some of our working hypotheses as to the rationale for these interactions. The suggestions have involved the functioning of living muscle since it is unlikely that the glycolytic enzymes participate in a programmed destruction of the muscle cell after death. It is my belief, however, that a full understanding of the functioning of glycolysis in muscle postmortem, and the changes dependent on glycolysis, requires a knowledge of the effects of the interaction of the enzymes and the particulate structures of the cell.
ACKNOWLEDGEMENTS

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REFERENCES


M. L. Greaser: What material are you using to bind your enzyme? You refer to it as particulate material.

H. O. Hultin: It has varied considerably depending on the particular enzyme. The best example is lactic dehydrogenase, which we have bound to a whole total cell homogenate, to actin, and to carboxymethylcellulose. You do get differences in the kinetic properties of the enzyme depending on the nature of the materials to which it binds, and we feel this is due principally to the charge characteristics of the binding surface.

M. L. Greaser: So you've been using mainly muscle homogenates and the particulate material after you've washed out substrates and so on?

H. O. Hultin: We take out very light fractions and very heavy fractions because of mechanical problems through the stop-flow. It is a mixture, containing most all of the components.

M. L. Greaser: Is it your idea, then, that the subcellular fractions that most people prepare by conventional methods contain a large amount of the glycolytic enzymes?

H. O. Hultin: We have done some work on this. As an example, we can prepare mitochondria which by the usual criteria seem to be in pretty good condition. If we feed them any of the phosphorylated sugars like glucose-6-phosphate, fructose-6-phosphate or fructose diphosphate, they perform as well in respiration as with succinate, so the glycolytic enzymes are not the limiting factor in the mitochondria. The exception is hexokinase, which is much slower if given glucose.

C. E. Allen: Could you give us a little more specific information on LDH-V in relation to temperature postmortem, where we get into quality problems?

H. O. Hultin: This is very difficult to summarize, and the best answer I can give is that it's unpredictable. For one of the iso-enzymes, LDH-1, there is no difference in activity between 23 and 40°, for LDH-111 it is intermediate, and for LDH-V the difference is very large. The effective difference between LDH-111 and LDH-V, which for the soluble forms is fairly significant, tends to disappear on binding, because binding doesn't affect LDH-111 very much but affects LDH-V very drastically.

C. E. Allen: Would you care to relate the temperature-dependent accumulation of calcium to postmortem cooling rate?

H. O. Hultin: The classical explanation of the cold-shortening effect is based on two features, one the ability of the sarcoplasmic reticulum fraction to maintain calcium in the bound state, the other the contractile apparatus itself. If these two have different temperature dependencies, there are going to be some temperatures at which one of the reactions dominates over the other, so that as you lower the temperature, say to 5°C, you can get a reversal in the relative importance of the two
systems. I would just like to throw in one comment, an idea which is not necessarily related to this question: we have seen with a fair number of enzymes that there is sometimes a very significant drop in the $K_m$ of the enzyme with temperature. This means that if the concentration of substrate in the muscle is of the order of magnitude of the $K_m$, which it most likely is, then as you lower the temperature you may not be lowering the activity of the enzyme. We see this a little bit with chicken breast muscle LDH, which has exactly the same activity at $40^\circ$ as at $4^\circ$ provided you use low pyruvate concentration. Now with high pyruvate concentration, you see an Arrhenius-type relationship, with about a seven-fold difference. But if you lower the substrate concentration down to what is most likely in the muscle, this difference disappears. It is amazing just how little work has been done on temperature effects on enzymes.

B. B. Marsh: Thank you very much, Herb, for a stimulating paper and an active discussion.

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H. F. Bernholdt: I want to welcome you to the Food Service Committee's session this afternoon and I want to thank in particular the Committee members that worked with us through the year that helped put this program together on the Challenges and Opportunities in the Food Service Industry. I am sure we all recognize the importance of the Food Service industry to the meat industry and to the food industry in general. With our mobile population, with the unique problems of feeding people on the move or in particular situations such as hospitals or institutions, it is a challenging area--it's a fast moving area. In fact, this afternoon it is so fast-moving that one of our speakers cancelled. The first speaker on the program was to be from the airline industry and unfortunately due to other priorities they had to cancel. However, I feel that in spite of that we have a well-rounded and full program that certainly will be of interest to all of us here. It is unfortunate that the airline people are not represented because theirs is a unique industry and their problems are unique. Certainly the logistics of feeding in an airline situation present many, many problems. I would recommend to future Food Service Committees that they consider again including this topic of airline feeding in their program. I would also suggest since they have expressed an interest that one of these speakers be from Sky Chefs in New York City who said that given a little time and a little opportunity they would be very, very happy to participate in the American Meat Science Association meeting. So I have very quickly dispatched of the airline industry and we will move on to our second topic--that of the institutional trade. We have with us today Mr. Bob Alstrin of Armour and Company. Bob is a graduate of Cornell University with a B.S. from the Hotel Restaurant Administration School and, of course, knows many of our friends there including our good and departed friend, Jerry Wanderstock. He was in the Navy as a pilot from 1956 to 1959. From 1959 to 1968, Bob was General Manager of several Stauffer Restaurants in Chicago and Cleveland. He was also Director of Restaurant Operations on a venture team with General Mills from 1968 to 1970. He is presently Sales Manager for Prepared Foods and Portion Control Meats for Armour and Company located in their Phoenix general office. In addition, he served on the Board of Directors of the Chicago Illinois Restaurant Association from 1967-1968. So we have with us a man who has long time experience in the food service industry and will talk to us today on "Institutional Feeding."