CCXXV. SULPHYDRYL GROUPS AND ENZYMIC OXIDO-REDUCTION[1]

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In an attempt to correlate the observable physical changes that occur within the cell at mitosis with definite chemical processes, the author was led to formulate the working hypothesis [Rapkine, 1931] that cell division is accompanied by configurational changes of the cell proteins resulting in an increase of protein SH groups.

When it was later shown that iodoacetic acid reacts stoichiometrically with SH derivatives like cysteine and reduced glutathione [Dickens, 1933, 1, 2; Rapkine, 1933, 1] as well as with protein SH [Rapkine, 1933, 2] according to the equation

$$R-SH+ICH_2COOH=HI+R-S-CH_2COOH,$$

various attempts were made [Ellis, 1933; Runnström, 1935; Rapkine, 1937] to study the effect of this halogen acid on cell division.

In the meantime results published by various authors not only strengthened the belief that sulphydryl proteins (reduced—partially denatured(?)—proteins) may play in some manner an essential part during mitosis, but also lent more likelihood to the supposition that whenever the activity of a biological system proves to be inhibited by iodoacetic acid it is because this acid acts on SH groups contained in this system. Such are the results obtained on the influence of temperature, *p*H and concentration on the velocity of interaction of iodoacetic acid and the SH groups of cysteine, glutathione (GSH) and denatured proteins [Rapkine, 1933, 1, 2; 1936]. Also the work of Schubert [1936] has shown that the speed of interaction of iodoacetic acid with tertiary amines is a good deal lower than with SH derivatives. Finally it was possible to show that after having inhibited cell division in yeast cells, by carefully controlled action of iodoacetic acid, these cells could be made to divide again by adding to the culture medium SH compounds [Rapkine, 1937].

It was therefore thought desirable that as many protein systems as possible should be studied *in vitro* with respect to their sensitiveness to iodoacetic acid. Those which should prove to be appreciably inactivated by this acid were to be examined for SH and to see to what extent their activity depended on the integrity of thiol groups within their structure. As prototypes of such systems enzyme preparations appeared to be the most adequate.

The choice of particular enzymes must perforce be an empirical one, since very little is known about those which may be involved in the metabolism of the cell during its division. The choice, however, as to the class of enzymes need not be, as far as our own work on cell division is concerned, empirical. The results obtained in the first paper of this series on the sea-urchin egg [Rapkine, 1931] led us, indeed, to think that the period of predivision is characterized by dehydrogenation processes. Moreover, the lactic acid production in the sea-urchin egg

[1] A preliminary note has already been published [Rapkine, 1938].

was correlated with the presence of SH groups. Our attention was therefore directed to enzymes which catalyse dehydrogenation.

This paper is concerned with the enzyme which catalyses the oxido-reduction between triosephosphate and pyruvate according to the following equation:

$$\text{Triosephosphate} + \text{pyruvate} \rightarrow \text{phosphoglycerate} + \text{lactate}.$$

The presence of coenzyme I is necessary for this oxido-reduction to take place [Meyerhof & Ohlmeyer, 1936; Euler *et al.* 1936]. A detailed study of the nature of the enzyme was made by Green *et al.* [1937] who, in the course of their work, have shown that it is one of a very small number of dehydrogenases that are readily inhibited by fairly low concentrations of iodoacetic acid.

Although there exists already an extensive literature dealing with the thiol groups of hydrolytic enzymes, and how the activity of these enzymes is influenced by oxidation and reduction of their SH groups,[1] the only work of this kind on dehydrogenases has recently been published by Hopkins & Morgan [1938] who brought forward evidence showing that succinic dehydrogenase depends for its activity on the presence of thiol groups.

In the work to be described in the present paper it will be shown that the activity of the enzyme responsible for the oxidation of triosephosphate by pyruvate depends on its state of oxidation-reduction. When oxidized by I_2 or by oxidized glutathione (GSSG) its activity is greatly diminished. When re-reduced by cysteine, reduced glutathione (GSH) or H_2S it is reactivated considerably. Oxidized methylene blue is shown to have no effect whatsoever. With the working hypothesis in mind that it is the SH of the enzyme proper that is being oxidized and reduced, further experiments were made with Cu_2O which is known to react particularly readily with thiol compounds [Hopkins, 1929; Pirie, 1931]. In this case too the evidence obtained shows that the activity of the enzyme can be nearly totally suppressed by Cu_2O and nearly fully regenerated by treatment with H_2S.

However, in order to ascertain that in the experimental conditions to be described the action of Cu_2O, as well as the action of oxidants and reductants, are not concerned rather with the coenzyme than with the enzyme, experiments were run in which the coenzyme was removed by adsorption before any subsequent treatment of the enzyme. The results obtained show that this is not the case; the action of Cu_2O, oxidants and reductants concerns mainly (if not entirely) certain active centres (SH) of the enzyme proper.

I. *Preparation of the enzyme and methods of estimating its activity*

The enzyme is essentially a dialysed aqueous extract of dried acetone powder from rabbit skeletal muscle. It is conveniently prepared by the technique of Meyerhof & Kiessling [1935] according to the directions of Green *et al.* [1937]. The method and the conditions of estimating enzymic activity were essentially the same as those used by the latter authors. Since the oxido-reduction of triosephosphate and pyruvic acid results in the production of acid, the activity of the enzyme which catalyses this reaction may be followed manometrically after addition of $NaHCO_3$ to the mixture containing the enzyme, the substrate and pyruvic acid. Since reduction of pyruvic acid to lactic acid involves no change in acidic groups, the increase in CO_2 pressure is due to phosphoglyceric acid which is formed on oxidation of triosephosphate. There is still however one more condition to be observed if the CO_2 production is to serve as a means of measuring the activity of the enzyme. Hexosediphosphate, which is used throughout these

[1] Full references are to be found in an excellent critical review by Hellerman [1937].

experiments as the source of triosephosphate, is known to give rise to pyruvic acid via phosphoglyceric acid. Fluoride inhibits this reaction, and it must therefore be added in all experiments. Measurements were carried out at 25° and at 30° in the presence or absence of O_2.

II. *Experimental procedure*

(*a*) *Experiments on inactivation of the enzyme by oxidizing with GSSG.* Preliminary experiments have shown that fairly concentrated solutions of GSSG were necessary and the following procedure was adopted: 5 ml. enzyme solution were placed with 2·5 ml. *M*/20 GSSG (final concentration of GSSG is *M*/60) in a Thunberg tube. The tube was evacuated and kept in the water bath at 30° for 3–5 hr. A control experiment was run along identical lines, in which the GSSG was replaced by 2·5 ml. water. The tubes were then opened and 1 or 1·5 ml. of each mixture was pipetted into a Warburg manometer cup. The following reagents were then added: 0·1 ml. *M* pyruvate, 0·3 ml. *M*/2 $NaHCO_3$, 0·7 ml. *M*/10 NaF, and finally 0·4 ml. *M*/10 hexosediphosphate was placed in the side-tube. After temperature equilibration, the hexosediphosphate was tipped from the side-tube into the main compartment of the Warburg cup and the gas evolution was followed.

(*b*) *Experiments on inactivation by oxidizing with I_2.* The action of I_2 is very rapid, and there was no necessity for incubating the enzyme with I_2 for more than 30 min. As the enzyme solution contains reducing substances which react immediately with I_2, it is quite impossible to know what fraction of the I_2 added reacts with the enzyme itself. The procedure adopted consisted in adding I_2 to an aliquot part of the enzyme solution until its reduction became slow. The amount varied with the enzyme preparation, the final I_2 concentration varying according to the experiments from 0·003 to 0·009 *N*. The rest of the procedure was exactly the same as in (*a*) above.

(*c*) *Experiments on inactivation with I_2 and GSSG, and reactivation with H_2S, GSH and cysteine.* When I_2 was used as the oxidizing agent, the mixture of enzyme and I_2, after keeping in the water bath at 30° for not more than 30 min., was divided into two equal parts, one of which was reserved, whereas the other was treated with H_2S. The H_2S was then eliminated by a current of H_2. As usual a third equal portion treated similarly except for the addition of I_2 served as control. Still another control of fresh enzyme solution was run for the manometric measurements, in order to see the extent of change in activity (if any) the enzyme may have suffered.

When GSSG was the inactivator, the enzyme solution, after incubation with GSSG at 30°, was dialysed for 15 hr. against distilled H_2O at 0° through a cellophane membrane. The enzyme solution was now divided up into three equal lots, *a*, *b* and *c*. To *a*, *M*/10 GSH was added to make the final concentration *M*/35. To *b* an equivalent amount of *M*/10 cysteine and to *c* the necessary amount of water were added. The three lots were now placed in Thunberg tubes; these were evacuated and placed in the water bath at 30° for 3–4 hr. A portion of the mutase solution, which served as a control, was taken through all the stages of treatment, dialysis, incubation, dilution etc., but without added glutathione. Dialysis of the enzyme solution results in a marked loss of activity, and therefore for the manometric measurements another control experiment was run with fresh untreated enzyme solution (adequately diluted) which had been kept in the ice chest at 0°.

One other technical detail deserves mention. Since prolonged dialysis results in a marked loss of activity, a second dialysis after treatment with GSH or

cysteine was not practicable. It is therefore necessary, whenever manometric measurements are done in the presence of O_2, to take into account the absorption of gas due to the oxidation of the excess GSH and cysteine, which in the presence of the enzyme solution is not negligible.

(*d*) *Experiments on inactivation with Cu_2O and reactivation with H_2S.* To 10 ml. enzyme solution 200 mg. Cu_2O were added. The mixture was kept in the water bath at 30° for 15–20 min., with occasional stirring. It was then filtered and divided into two equal parts. One was reserved, while the other was treated with H_2S in the usual manner. As a control a third portion underwent the same treatment (incubation and H_2S) but without addition of Cu_2O. In most experiments however the control portion of enzyme solution was taken through the stage of incubation at 30° only, since experience has shown that treatment with H_2S does not in any way change the activity of the enzyme.

(*e*) *Experiments on inactivation and reactivation of the enzyme after treatment with charcoal to remove coenzyme I.* Merck "Medicinal" charcoal proved to be a good adsorbent of coenzyme I, provided that the charcoal was first washed with distilled water until the wash-water was no longer acid. The enzyme loses most of its activity after two charcoal adsorptions only. On addition of coenzyme I[1] the activity of the enzyme solution is fairly completely restored. Once the enzyme had been charcoal-treated, it was taken through all the stages of inactivation with GSSG, I_2 and Cu_2O. Reactivation experiments were only attempted on charcoal-treated enzyme which had been inactivated by I_2 and Cu_2O. Further experimental details will receive mention in the description of the particular experiments.

III. *Results obtained*

(*a*) *Oxidation and inactivation by GSSG.* Table I shows the results obtained in typical experiments when the enzyme is incubated with GSSG at various concentrations and for various periods of time at 30°.

Table I

After incubation the following amounts of the reagents were pipetted into the Warburg manometer cups for manometric measurements of the enzymic activity; 1·5 ml. treated or untreated enzyme, 0·4 ml. *M*/10 hexosediphosphate, 0·1 ml. *M* pyruvate, 0·3 ml. *M*/2 $NaHCO_3$, 0·7 ml. *M*/10 NaF. The hexosediphosphate was in the side-bulb of the cup and was only tipped into the main compartment after temperature equilibration. The amounts and concentration of the reagents added were, unless otherwise stated, always the same and need not afterwards be repeated.

Final conc. GSSG	Time of incubation hr.	Temp. of manometric measurements		μl. CO_2 in 30 min.
M/100	1½	25°	Enzyme + GSSG	171
			Control without GSSG	256
M/100	1	25°	Enzyme + GSSG	160
			Control	198
M/60	5½	25°	Enzyme + GSSG	11
			Control	157
			Fresh enzyme	146
M/60	5½	25°	Enzyme + GSSG	17
			Control	137
			Fresh enzyme	145
M/60	4½	30°	Enzyme + GSSG	45
			Control	102
			Fresh enzyme	111

[1] I am indebted to Dr T. Cahn and Dr J. Houget for samples of coenzyme I.

As is to be noted, fairly high concentrations of GSSG and rather long periods of incubation are necessary in order to oxidize and hence to inactivate the enzyme. The control which has been put through all the stages of treatment except for the added glutathione, suffers very little loss of activity as can be seen on comparing its activity with that of fresh mutase which had been standing in the ice chest at 0° until the manometric measurements were made.

(*b*) *Oxidation and inactivation by I_2.* I_2 reacts very quickly with the enzyme and the time factor is unimportant. As is seen in Table II it is the I_2 concentration which matters.

Table II

Vol. enzyme ml.	Final conc. I_2	Time of incubation at 30° (min.)	Temp. of manometric measurements		μl. CO_2 in 30 min.
1·2	0·0009 *N*	30	25°	Enzyme + I_2	137
				Control	256
1·3	0·003 *N*	10	25°	Enzyme + I_2	72
				Control	183
1·0	0·009 *N*	30	25°	Enzyme + I_2	40
				Control	198

(*c*) *The action of methylene blue on the enzyme.* It was desirable to know whether some other oxidant than GSSG and I_2 could be made to bring about an inactivation of the enzyme. Oxidized methylene blue was tried. Methylene blue (0·006 *M*) was added to the enzyme solution until there was no more reduction, even after the mixture was evacuated in Thunberg tubes. After 3 hr. incubation in the water bath at 30°, the necessary reagents were added and the activity of the methylene blue-treated enzyme was compared with a control (Table III). No inactivation whatsoever occurred.

Table III

Manometric measurements at 25°.

	μl. CO_2 in 10 min.
Enzyme + methylene blue	106
Control without methylene blue	110

(*d*) *Inactivation with GSSG and reactivation with GSH and cysteine.* As was mentioned in section II, reactivation of GSSG-treated enzyme entailed the elimination of excess GSSG by dialysis which in itself caused a loss of activity of the enzyme. The figures in Table IV illustrate this, as well as the superiority of cysteine over GSH in reactivating the enzyme. This has been met with throughout all the experiments.

Table IV

Incubated with GSSG for 5 hr. Dialysed for 15 hr. at 0°. Incubated with GSH and cysteine for 3½ hr. Manometric measurements at 25°.

	μl. CO_2 in 30 min.
Enzyme oxidized by *M*/60 GSSG, then dialysed	37
Oxidized, dialysed enzyme reduced by *M*/35 cysteine	159
Oxidized, dialysed enzyme reduced by *M*/35 G-SH	81
Control, dialysed	95
Fresh enzyme diluted suitably	127

(*e*) *Inactivation with I_2 and reactivation with H_2S.* As long as I_2 is made to act upon a muscle extract containing both enzyme and coenzyme, the inactivation is very marked, but the reactivation is rather feeble (Table V).

Table V

Incubated at 30° for 10 min. Final concentration of I_2 0·003 *N*. Measurements at 25°.

	μl. CO_2 in 20 min.
Enzyme + I_2	23
I_2-treated enzyme reduced by H_2S	45
Control	88

As will be shown below, the reactivation by H_2S may be nearly complete if the I_2 is made to act upon an enzyme solution from which coenzyme I has been removed by charcoal adsorption.

(*f*) *Inactivation by Cu_2O and reactivation by H_2S.* Although inactivation by Cu_2O is rapid and thorough, the time of contact and the amount of Cu_2O are, up to certain limits, not negligible (Table VI). The degree of reactivation of

Table VI

Enzyme in contact for 3 min. at 30° with Cu_2O before filtration of excess of the oxide. 50 mg. Cu_2O per 5 ml. enzyme solution. H_2S was passed for 5 min.

	μl. CO_2 in 30 min.
Enzyme + Cu_2O	85
Cu_2O treated enzyme decomposed by H_2S	160
Control (treated with H_2S)	257

Enzyme in contact for 20 min. at 30° with Cu_2O before filtration. 100 mg. Cu_2O per 5 ml. enzyme. H_2S passed for 15 min.

	μl. CO_2 in 30 min.
Enzyme + Cu_2O	74
Cu_2O-treated enzyme decomposed by H_2S	243
Control (not treated with H_2S)	268

Cu_2O-treated enzyme by H_2S also varies with time, and it was found that 15–20 min. were quite sufficient for optimum reactivation.

(*g*) *Inactivation and reactivation of enzyme after treatment with charcoal to remove coenzyme I.* The fact that coenzyme I is in some methods of its preparation precipitated as a Cu^+ salt, albeit in acid solution, served as a warning in the interpretation of the results obtained when Cu_2O is made to act upon muscle extracts containing both enzyme and coenzyme I. Is not the inactivation of an enzyme solution by Cu_2O due mainly to its combination with coenzyme I?

It was at once clear that the only way to answer this question, not only for Cu_2O, but also for the other inactivators, was to subject the enzyme to oxidation and reduction after removal of its coenzyme by charcoal adsorption. Preliminary experiments showed to what extent the enzyme suffers loss of activity on adsorption (Table VII). It is certainly not negligible, and the addition of coenzyme I in varying amounts restores, at best, about 80 % of the original activity. As for the residual CO_2 production by the complete system in presence of charcoal-treated enzyme without added coenzyme I, it cannot be due to dismutation of triosephosphate, since in the presence of pyruvic acid dismutation is completely

suppressed [Green *et al.* 1937]. It is very likely due to remaining traces of coenzyme I which have escaped adsorption, and which allow some oxido-reduction between triosephosphate and pyruvate to take place.

Table VII

Manometric measurements at 25°.

	mg. cozymase added		
	1·5	1·0	0·5
	μl. CO_2 in 30 min.		
	(1)	(2)	(3)
Charcoal-treated enzyme + hexosediphosphate + pyruvate + NaF	33	29	55
As above but with coenzyme I	126	130	146
Complete system with untreated enzyme	153	195	178

The results of Table VIII show clearly that under the experimental conditions described the action of Cu_2O cannot be exerted mainly on coenzyme I. If it were through some action on coenzyme I that inactivation of the enzyme by Cu_2O

Table VIII. *Action of Cu_2O on charcoal-treated enzyme*

Charcoal-treated enzyme solution was divided into 7 portions. To portions *a*, *b*, *c* and *d* Cu_2O was added (100 mg. per 5 ml.). After Cu_2O treatment (20 min. at 30°) the excess Cu_2O was filtered off, and whilst *a* and *b* were reserved, *c* and *d* were treated with H_2S. Coenzyme I was now added to *a* and *c*. To portion *e* was added a sample of coenzyme I which had been treated with Cu_2O, and the excess of the latter filtered off. To *f* ordinary coenzyme I was added, whilst no coenzyme was added to *g*. A normal enzyme solution (not charcoal-treated) was now divided into four other portions, *h*, *i*, *j* and *k*. *h* was the normal control; *i*, *j* and *k* were treated with Cu_2O (20 min. at 30°), and after filtration from excess Cu_2O *k* was reserved, whilst coenzyme I was added to *i*. To *j*, instead of coenzyme I, fresh enzyme solution (enzyme + coenzyme) was added. Of each of these various portions of enzyme 1 ml. was pipetted into Warburg manometer cups, and the necessary reagents were added for manometric measurements (see part (*a*) of this section).

	μl. CO_2 in 30 min.
(*a*) Charcoal-treated enzyme + Cu_2O + coenzyme I	8
(*b*) As above but without coenzyme I	19
(*c*) Charcoal-treated enzyme + Cu_2O + H_2S + coenzyme I	102
(*d*) As above without coenzyme I	51
(*e*) Charcoal-treated enzyme + coenzyme I which has been treated with Cu_2O*	99
(*f*) Charcoal-treated enzyme + coenzyme I	170
(*g*) As above without coenzyme I	84
(*h*) Normal (not charcoal-treated) enzyme	183
(*i*) Normal enzyme + Cu_2O + coenzyme I	40
(*j*) Normal enzyme + Cu_2O + fresh enzyme†	197
(*k*) Normal enzyme + Cu_2O	45

* Coenzyme solution was in contact with Cu_2O (20 mg. per ml. for 20 min. at 30°).
† 0·5 ml. of fresh normal enzyme was added.

occurred, then we should expect the charcoal-treated enzyme to be reactivated, even after Cu_2O treatment, by mere addition of coenzyme I to the system. As is shown from Exps. *a* and *b*, this is not the case. Reactivation is only possible when the enzyme has been treated with H_2S (see Exp. *c* and *d*, Table VIII).

That Cu_2O does not react with coenzyme to any appreciable extent is also shown from *e*, where Cu_2O-treated coenzyme I was added to charcoal-treated

enzyme. Further evidence that Cu_2O acts mainly on some active centres of the enzyme proper is shown from Exp. *h*, *i*, *j* and *k*.

Action of I_2 on charcoal-treated enzyme. It was shown in part *e* of this section, that reactivation by H_2S of I_2-treated enzyme is rather small, so long as the I_2 is made to act upon ordinary enzyme solution. As will be seen from Table IX

Table IX

Final concentration of I_2 0·006 *N*. Manometric measurements at 25°.

	μl. CO_2 in 7 min.
Charcoal-treated enzyme + I_2 + coenzyme I	50
As above but without coenzyme I	49
Charcoal-treated enzyme + I_2 + H_2S + coenzyme I	113
Fresh mutase equally diluted	122
As above + I_2	29

good reactivation may be obtained if the I_2 is made to act upon an enzyme solution from which coenzyme I is removed. Moreover, it seems that the inactivation by I_2 of normal enzyme solution is more pronounced than in the case of charcoal-treated enzyme.

Action of GSSG on charcoal-treated enzyme. The results of Table X show the action of GSSG on charcoal-treated enzyme as compared with its action on normal enzyme (untreated with charcoal). The action of GSSG is much more

Table X

The enzyme was incubated with GSSG (final concentration *M*/60) for 4 hr.

	μl. CO_2 in 27 min.
Normal enzyme suitably diluted	228
As above + GSSG	181
Charcoal-treated enzyme + GSSG + coenzyme I	58
As above but without coenzyme I	18
Charcoal-treated enzyme + coenzyme I	147
As above but without coenzyme I	52

pronounced on charcoal-treated enzyme. It must be said that at present no explanation has been found to account for the difference of action of either I_2 or GSSG on normal and charcoal-treated enzyme preparations. Further work is being done in this direction.

Discussion

The strict interpretation of the experiments described is that the enzyme responsible for the catalysis of the oxido-reduction between triosephosphate and pyruvic acid depends for its activity on its own oxidation-reduction state. Acted upon by oxidants, the activity of the enzyme is greatly diminished or nearly suppressed; once inactivated, the enzyme may recover its activity to a great extent by controlled treatment with reductants. In making the tentative hypothesis that these changes in activity of the enzyme are to be ascribed to the oxidation and reduction of the SH groups of the enzyme, one must necessarily cope with the long-standing objections which have already been formulated in the case of hydrolytic enzymes. To begin with, are the thiol groups involved at all? If they are, might there not be, side by side with these, other groups which are at least as essential for the activity of the enzyme? It is, of course, impossible at present to state definitely that there are no other active groups than the

thiol groups in the enzyme studied here. However, the hypothesis that thiol groups are mainly responsible for the activity of the enzyme can be supported by many arguments. Iodoacetic acid inhibits the enzyme readily and thoroughly. Although this acid has been shown to react with other groups, the speed with which it reacts with SH confers on this halogen acid, for practical purposes at any rate, the property of specificity towards thiol groups. The same can virtually be said of Cu_2O, in view of the conditions under which this acts upon the enzyme preparation. The only difference is that an enzyme completely inactivated by Cu_2O can be fully reactivated by H_2S, whilst inactivation by iodoacetic acid is irreversible.

In connexion with the reversible inactivation by GSSG, the arguments brought forward by Hopkins & Morgan [1938] in the case of succinic dehydrogenase are relevant. GSSG is a very mild oxidizing agent, and the fact that the GSSG $\rightleftharpoons$ 2GSH system is very negative in the potential scale makes it very likely that, as an oxidant for proteins, GSSG is specific for the thiol groups of the protein. There is further evidence for this. Hopkins [1925] has shown that in the oxidation of the reduced thermostable residue of tissue by GSSG, the resulting GSH formed is a measure of the pre-existing SH of the proteins. This was later confirmed on reduced heat-coagulated egg-white [Rapkine 1933], where it was, moreover, shown that the amount of pre-existing fixed SH of the protein as measured by the iodoacetic acid technique is identical with that obtained by Hopkins's method. Finally, Mirsky and Anson [1935] proved conclusively that the total number of fixed SH groups in a protein can be approximately measured by the total amount of hydrogen transferred to the oxidant (cystine).

The action of oxidized methylene blue on the enzyme studied here is again in harmony with the thiol group hypothesis. Methylene blue is, like GSSG, a mild oxidizing agent, and although it is high up in the scale of redox potentials it is known to react extremely slowly with thiol groups. This accounts for the ineffectiveness of methylene blue in inactivating the enzyme.

The action of I_2 needs hardly any comment. I_2 is by no means a mild oxidizing agent. Although it oxidizes SH to SS it cannot be said that it reacts readily only with SH groups. The action of I_2 on the enzyme must therefore be considered only in conjunction with the action of other substances such as GSSG, methylene blue and Cu_2O. However, it must be pointed out that inactivation of the enzyme by I_2 is to a large extent reversible by H_2S, especially if the I_2 is made to act on a coenzyme-free (charcoal-treated) preparation of the enzyme.

The behaviour of GSSG towards charcoal-treated enzyme is rather curious. Unless other substances present in the normal enzyme preparations interfere in some manner with the action of GSSG, it is perhaps not unreasonable to think that the coenzyme exerts in some way some protective influence on the enzyme. Once the coenzyme is removed by charcoal, GSSG inactivates the enzyme much more quickly. Whatever the explanation may be, account should be taken of these results whenever similar experiments of oxidation and reduction are carried out on enzymes which require, for their activity, the presence of a coenzyme.

This paper deals with the reversible inhibition of an enzyme which takes part in the first stages of glycolysis. It is therefore not out of place to compare the results obtained on this particular enzyme with those obtained previously by other authors on the effect of inhibitors on glycolysis by whole muscle extracts. Thus Lipmann [1934] showed that Cu in small amounts (10^{-5} to $10^{-4}M$) activates muscle glycolysis, whilst in concentrations of 10^{-4} to $10^{-3}M$ it inhibits

glycolysis. Previously the same author [Lipmann, 1933] had shown that the inhibition of glycolysis by dichlorophenol-indophenol and O_2 could be removed by ascorbic acid and N_2.

Wagner-Jauregg & Rzeppa [1936, 1, 2] obtained evidence that the copper inhibition described by Lipmann [1934] may be reversed by GSH, cysteine, coenzyme I and coenzyme II. More recently a detailed systematic study on the reversible inhibition of muscle glycolysis was made by Gemmill & Hellerman [1937]. Besides metallic compounds the authors also used I_2 as an inactivator of glycolysis, the reactivants used being cysteine, GSH and ascorbic acid. In this same paper [Gemmill & Hellerman, 1937] mention is made of unpublished experiments by Leövey (carried out in 1936) in which the author shows that the conversion of pyruvate into lactate in rabbit muscle pulp is inhibited by I_2 and quinone and restored by H_2S.

The results just enumerated make it tempting indeed to suggest that precisely the enzymic system dealt with in the present paper is one of the systems with which the above-mentioned researches on glycolysis was concerned.

Summary

1. The enzyme which catalyses the oxido-reduction between triosephosphate and pyruvate is inactivated by oxidation with GSSG. If now the excess GSSG is dialysed away, the enzyme can be reactivated by GSH or cysteine, the latter being more rapid in its action.

2. The enzyme is inactivated by I_2. Such an inactivated enzyme can, to a large extent, be reactivated by H_2S. The degree of reactivation is greater if the I_2 is made to act upon an enzyme preparation from which coenzyme has been removed by charcoal adsorption.

3. Methylene blue exerts no influence on the activity of the enzyme.

4. Cuprous oxide completely inactivates the enzyme. Its activity can, however, be fully recovered on regenerating the enzyme by H_2S.

5. It is shown, using charcoal-treated enzyme preparations, that the action of Cu_2O, oxidants and reductants is mainly concerned with active groups of the enzyme itself.

6. The changes in activity of the enzyme under the influence of Cu_2O, oxidants and reductants are ascribed mainly to SH groups. The oxidation or combination of these groups results in an inactivation of the enzyme. The re-reduction or regeneration results in a reactivation of the enzyme. This hypothesis is discussed.

7. The work just reported is discussed in the light of work done by other authors on glycolysing extracts.

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