CLX. COENZYME FACTOR OF YEAST

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Animal tissues have been shown to contain coenzyme factor, an enzyme which catalyses the oxidation of coenzymes I and II by carriers [cf. Dewan & Green, 1937; 1938; Adler *et al.* 1937]. In this communication evidence is presented of the existence of the same enzyme in baker's yeast and other micro-organisms. The wide distribution of coenzyme factor offers additional evidence that it is the normal physiological agent for catalysing the oxidation of the pyridine nucleotides.

I. *Preparation of yeast coenzyme factor*

There are two methods available for extracting enzymes from yeast. The first and standard method is to dry yeast slowly, resuspend in water and allow autofermentation to proceed for some hours. By this procedure the more stable enzymes can be extracted. The method however is not applicable to the fragile enzymes like coenzyme factor and cytochrome oxidase which are destroyed in the course of drying the yeast. A more suitable method for obtaining these enzymes is that of the wet crushing mill (for a detailed description of the mill, cf. Booth & Green [1938]).

A suspension of baker's yeast (1 part yeast cake to 1 part water) was thoroughly ground in the roller mill and then centrifuged for 20 min. The supernatant liquid was mixed with ½ vol. saturated $(NH_4)_2SO_4$ (pH 5) and was centrifuged. The supernatant fluid was discarded and the sediment was redissolved in the original volume of water. The precipitation with $(NH_4)_2SO_4$ was repeated twice. The precipitate was finally dissolved in the original volume of phosphate buffer, pH 7·2. By this procedure the coenzyme factor was obtained free from the soluble coenzyme dehydrogenases and from flavoprotein. Like its counterpart in animal tissues the yeast coenzyme factor is associated with highly peptized insoluble particles. As purification proceeds the insolubility of the particles becomes more noticeable. Sedimentation of the particles after the $(NH_4)_2SO_4$ procedure becomes feasible even in salt-free solution.

II. *The catalytic effect of yeast coenzyme factor on animal coenzyme dehydrogenase systems*

The coenzyme dehydrogenases catalyse the oxidation of their respective substrates by one of the two pyridine nucleotides. The reduced coenzyme is non-autoxidizable and its reaction with O_2 can only take place through the intermediation of some natural carrier such as cytochrome *a* or *b*, or an artificial carrier such as methylene blue. Coenzyme factor catalyses the reaction between reduced coenzyme I or II and the carrier. A system containing dehydrogenase, substrate, coenzyme, carrier and O_2 will not absorb O_2 unless coenzyme factor is added. Such a system is obviously suitable for testing the catalytic effect of coenzyme factor.

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The preparation of the lactic, malic and triosephosphoric dehydrogenases of animal source free from coenzyme factor has already been described by Dewan & Green [1938]. Table I shows the effect of yeast coenzyme factor on these three systems. We have also been able to demonstrate the factor effect in the hexosemonophosphoric dehydrogenase system of rabbit skeletal muscle. Our colleague H. S. Corran will deal with the action of coenzyme factor on this system in a separate communication.

Table I. *The catalytic effect of yeast coenzyme factor on the lactic, triosephosphoric and malic dehydrogenase systems of animal tissues*

	μl. O_2 per 10 min.		
	Lactic	Malic	Triose-phosphoric
Complete system	189	181	165
Without factor	9	10	0
Without coenzyme	0	0	0
Without substrate	5	0	21
Without dehydrogenase	3	6	0

The following quantities were used in the complete systems; *lactic*—1·5 ml. dehydrogenase, 0·2 ml. factor, 0·7 ml. 0·2% coenzyme I, 0·2 ml. 2 *M* HCN, 0·05 ml. 0·5% methylene blue, 0·3 ml. *M*/3 pyrophosphate and 0·2 ml. *M* lactate; *malic*—same quantities except 0·2 ml. *M* malate instead of lactate; *triosephosphoric*—1·5 ml. dehydrogenase, 1 ml. factor, 0·7 ml. 0·2% coenzyme I, 0·2 ml. 0·5% methylene blue, 0·3 ml. *M*/3 pyrophosphate and 0·4 ml. *M*/10 hexosediphosphate (KOH in the centre pot).

III. *The catalytic effect of yeast coenzyme factor on yeast coenzyme dehydrogenase systems*

The malic, alcohol and triosephosphoric dehydrogenases were prepared from yeast by the following procedures.

Malic. The centrifuged juice of crushed baker's yeast was mixed at 0° with 3 vol. cold acetone. The precipitate was filtered and washed with acetone and ether. The dried powder was rubbed up with the original volume of water and the suspension was dialysed for 12 hr. at 0°. The precipitate was centrifuged and discarded. The solution was then treated for 10 min. at 52°.

Alcohol. Maceration juice of top brewer's yeast was diluted with 2 vol. water and dialysed for 6 hr. against running tap water. The centrifuged solution was then made 0·6 saturated with respect to $(NH_4)_2SO_4$, the precipitate collected by centrifuging and redissolved in the original volume of water. The $(NH_4)_2SO_4$ precipitation was repeated twice. The final solution was treated at 0° with an equal volume of cold acetone, and the precipitate was filtered and washed with acetone and ether. The dried powder was finally dissolved in the original volume of water.

Triosephosphoric. Dried baker's yeast was rubbed up with 10 vol. iced water and the suspension was centrifuged. This washing procedure was thrice repeated. The final suspension was made up by mixing 1 vol. of washed and centrifuged yeast cake with 2 vol. of water.

These three preparations contain highly active dehydrogenases free of coenzyme factor and flavoprotein. Table II shows the effect of addition of coenzyme factor to these dehydrogenase systems. It is clear that regardless of the source of the coenzyme dehydrogenase, whether from animal tissues or yeast, the reaction of reduced coenzyme I with methylene blue (or the natural carriers)

Table II. *The catalytic effect of yeast coenzyme factor on the malic, alcohol and triosephosphoric dehydrogenase systems of yeast*

	μl. O_2 per 15 min.		
	Malic	Alcohol	Triose-phosphoric
Complete system	212	134	235
Without factor	29	17	26
Without coenzyme	0	0	10
Without substrate	0	0	22
Without dehydrogenase	0	0	0

The following quantities were used in the complete systems: *malic*—1·5 ml. dehydrogenase, 0·2 ml. factor, 0·7 ml. 0·2% coenzyme I, 0·2 ml. 2*M* HCN, 0·2 ml. 0·5% methylene blue, 0·3 ml. *M*/3 pyrophosphate and 0·2 ml. *M* malate; *alcohol*—1·5 ml. dehydrogenase, 0·5 ml. factor, 0·5 ml. 0·2% coenzyme I, 0·2 ml. 0·5% methylene blue, 0·3 ml. *M*/3 pyrophosphate, 0·2 ml. *M*/2 hydrazine and 0·1 ml. 98% alcohol; *triosephosphoric*—2 ml. yeast suspension, 0·5 ml. factor, 0·5 ml. 0·2% coenzyme I, 0·4 ml. *M*/10 hexosediphosphate and 0·3 ml. *M*/3 pyrophosphate (KOH in the centre pot).

takes place only in presence of the coenzyme factor. Furthermore, yeast factor works as efficiently with animal coenzyme dehydrogenase systems as does animal factor, and *vice versa*.

IV. *Comparison of yeast factor and flavoprotein*

Dewan & Green [1938] have shown that 1 mg. crude coenzyme factor of pig heart has the same activity as 20 mg. pure flavoprotein in the malic system (pig heart) and as 70 mg. pure flavoprotein in the triosephosphoric system (rabbit muscle). Tests with the crude yeast factor show that 1 mg. dry weight has the same activity as 6 mg. pure flavoprotein in the malic system (pig heart) and as 20 mg. pure flavoprotein in the triosephosphoric system (rabbit muscle). This discrepancy in no wise indicates that the coenzyme factors from the two sources are not the same. In the next section evidence is presented of the extraordinary similarity in properties of the enzymes from the two sources. It is important to note that the crude preparations of the factor whether from animal tissues or yeast contain all the insoluble enzymes and particles extracted from the cell. The coenzyme factor probably accounts for much less than 1% of the total dry weight. At this level of purity the absolute activity of the coenzyme factor is not very significant in a comparison of the enzymes prepared from different sources. What is significant however is that the catalytic activity of even the crude coenzyme factor is incomparably higher than that of pure flavoprotein.

The crude preparations of yeast factor were tested for the presence of flavoprotein with negative results. The fact that weight for weight the crude yeast coenzyme factor is many times more active than pure flavoprotein rules out all possibility that traces of flavoprotein could account for the catalytic effects. Flavoprotein was prepared from brewer's yeast by the method of Warburg & Christian [1932]. The flavin content was determined by extracting the dried powder with aqueous methyl alcohol and estimating the flavin content of the extract.

V. *Some properties of the yeast coenzyme factor*

Table III shows the extraordinary fragility of the yeast coenzyme factor. All these properties are identical with those of the coenzyme factor of pig heart. The yeast factor is also partially destroyed by drying and by precipitation with acetone.

Table III. *The effect of reagents and temperature on the activity of the yeast coenzyme factor*

Treatment	% loss in activity
10 min. at 52°	92
1 hr. at 38°	50
20 min. digestion with trypsin at 38°	100
3 min. at *p*H 4·0	75
3 min. at *p*H 4·6	0
3 min. at *p*H 9·0	90
12 hr. dialysis at 0°	10

Thus far we have been unable to detect any colour characteristic of the suspension of the coenzyme factor. The more concentrated suspensions are slightly brown but this colour does not change when the complete catalytic system is functioning. Dr Straub of the Molteno Institute kindly tested our preparations of the coenzyme factor for the presence of the coenzyme of the *d*-amino-acid oxidase. Only minute amounts were found to be present. As yet no direct evidence is available for assuming that the coenzyme factor contains the flavin-adenine coenzyme as its prosthetic group. Numerous attempts were made to resolve the coenzyme factor into a protein and crystalloidal component, but in no case was there evidence of such a resolution.

VI. *Coenzyme factor in other micro-organisms*

By a procedure similar to that employed in the case of baker's yeast and by the use of the roller mill it was possible to demonstrate the presence of coenzyme factor in the following micro-organisms: *Bact. coli*, *Bact. proteus* and *Bact. subtilis*. The most active preparation was obtained from *Bact. coli*.

Summary

1. The preparation of coenzyme factor from crushed suspensions of baker's yeast and other micro-organisms is described.

2. Like its counterpart in animal tissues the enzyme catalyses the oxidation by carriers of the reduced diphosphopyridine nucleotide (coenzyme I).

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