PRELIMINARY NOTES

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Isolation of high-molecular-weight NADH dehydrogenase of the respiratory chain by diethyl ether – Triton X-100 treatment

All hitherto isolated preparations of NADH dehydrogenase of the respiratory chain can be divided, according to SLATER[1], into two types—a high-molecular-weight type (I) and a low-molecular-weight type (II). The main differences between the two types, apart from the molecular weights, are: (1) acceptor specificity; (2) substrate-reducible ESR signal at $g = 1.94$; (3) Fe:FMN ratio; (4) temperature-dependent inactivation by –SH reagents; (5) inactivation by high NADH concentration; (6) stability; (7) transformation of Type I into Type II by different agents. While Type I dehydrogenase has been isolated only by a single procedure, *i.e.*, by the digestion of the respiratory particles with phospholipase at 30° (ref. 2), Type II has been isolated[3–5] both by the acid–ethanol extraction method of MAHLER *et al.*[6] and by digestion with phospholipase at 37° (ref. 7).

We wish to report here that an enzyme with several properties characteristic of SINGER's 30° enzyme can be isolated from ether-pretreated particles[8] by means of the non-ionic detergent, Triton X-100.

Beef heart muscle preparation was obtained by differential centrifugation at neutral pH as described by SLATER[9]. The preparation was treated with diethyl ether for 15 min and after centrifugation the solvent was decanted and evaporated. The particles were resuspended in 50 mM phosphate buffer. The suspension was adjusted to pH 8.5 with ammonia and extracted at 30° with 1 % Triton X-100 for 1 h. The yellow supernatant fraction was obtained for assays after two centrifugations, the first at 40000 $\times g$ for 40 min and the second at 105000 $\times g$ for 2 h. Ferricyanide reductase activity

TABLE I

ACCEPTOR SPECIFICITY OF NADH DEHYDROGENASE

Assay conditions of enzymic activities at fixed concentration of electron acceptor have been described in a previous paper[8]. The activity of lipoamide dehydrogenase was determined by the method of MASSEY AND VEEGER[11]. The v_{max} of NADH-ferricyanide activity was measured in triethanolamine buffer (pH 7.8) according to the standard procedure of RINGLER, MINAKAMI AND SINGER[2].

Electron acceptor	*Specific activity*		*Relative rate*	
	At fixed concn. of acceptor	*At v_{max}*	*Present enzyme*	*Singer's enzyme**
$K_3Fe(CN)_6$	17.4	170	100	100
Cytochrome *c*	0.087		0.5	0.3
Menadione	0.180		1.4	0.9
2,6-Dichlorophenolindophenol	0.530		3.1	3.0
Lipoic acid	0.042		0.2	0.4**
Q-1	0.135		0.8	

* Partialy purified enzyme with specific activity 200 (v_{max}) (ref. 10).
** Lipoamide assay.

recovered in the first supernatant fraction amounted to 60–75 % of that in the ether-pretreated preparation. In the absence of detergent, the enzyme tended to aggregate like SINGER's purified enzyme which is soluble only in the presence of 0.5 % Triton X-100 (ref. 2). When frozen, the preparation can be stored for weeks without loss of ferricyanide activity.

Table I shows the acceptor specificity of NADH dehydrogenase together with comparable data of relative rates for SINGER's partially purified enzyme[10] of approximately the same specific activity. As seen from Table I, both preparations displayed essentially a single activity, *i.e.*, ferricyanide activity. Activities measured with other acceptors were negligible.

The –SH groups of SINGER's 30° enzyme are relatively resistant to the action of mercurials at low temperature. However a temperature-dependent inactivation by –SH reagents has been observed particularly in the presence of triethanolamine buffer[12]. Table II shows essentially identical properties of the NADH dehydrogenase isolated by Triton X-100 treatment. However the inhibition of ferricyanide reductase activity was much lower.

TABLE II

EFFECT OF TEMPERATURE AND OF PHOSPHATE IONS ON INACTIVATION OF NADH DEHYDROGENASE BY *p*-CHLOROMERCURIBENZOATE

The preparation was incubated for 5 min as described by CREMONA AND KEARNEY[12].

Buffer	*Temp. of incubation*	*Concn. of p-chloromercuri-benzoate (M)*	*Inactivation (%)*
Phosphate	0	$1 \cdot 10^{-5}$	0
		$3.3 \cdot 10^{-5}$	0
	30	$1 \cdot 10^{-5}$	0
		$1 \cdot 10^{-4}$	14
Triethanolamine	0	$1 \cdot 10^{-5}$	0
		$3.3 \cdot 10^{-5}$	10
	30	$1 \cdot 10^{-5}$	25
		$1 \cdot 10^{-4}$	30

The capability of the high-molecular-weight dehydrogenase to undergo transformation is a well-known property of the enzyme. The transformation results in the emergence of cytochrome *c* and 2,6-dichlorophenolindophenol activities[13]. Fig. 1 presents the transformation of NADH dehydrogenase with ferricyanide activity into cytochrome *c* and Q-1 reductase activity. The decrease of the ferricyanide activity was accompanied by a 17- and 10-fold elevation of cytochrome *c* and Q-1 activities, respectively.

Inactivation of NADH dehydrogenase induced by incubation with reduced substrate is another characteristic of this enzyme[14]. Fig. 2 shows the inactivation of ferricyanide activity of the present enzyme following incubation with NADH and NADPH.

The procedure described for the isolation of Type I dehydrogenase might provide additional support for the often-questioned theory of the existence in the respiratory chain of a high-molecular-weight enzyme which comprises, or from which are derived, cytochrome *c* and Q-1 reductases.

Purification and further characterization of the enzyme are now being undertaken.

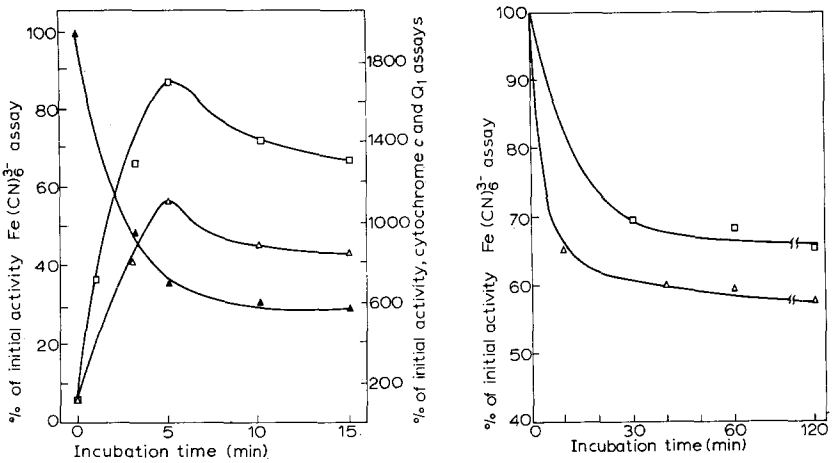

Fig. 1. Transformation of NADH dehydrogenase into NADH cytochrome *c* and Q-1 reductases. The preparation was incubated at 34° in the presence of 9% ethanol at pH 6.0 for the time indicated. Then the suspension was chilled, neutralized to pH 7.4 and the precipitated protein was removed by centrifugation. ▲—▲, $Fe(CN)_6^{3-}$; △—△, Q-1; □—□, cytochrome *c*.

Fig. 2. Inactivation of NADH dehydrogenase following incubation with NADH (△—△) and NADPH (□—□). The preparation was incubated in phosphate buffer essentially as described by Rossi *et al.*[14] with 5 mM NADH or NADPH in air at 22°.

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