BBA 45826

EFFECT OF SUBSTRATES ON RECONSTITUTION OF THE MITOCHONDRIAL RESPIRATORY CHAIN UNDER VARIOUS CONDITIONS

V. N. LUZIKOV, M. M. RAKHIMOV AND I. V. BEREZIN
Laboratory of Bioorganic Chemistry, Moscow State University, Moscow (U.S.S.R.)
(Received February 17th, 1969)

SUMMARY

The reconstitution of NADH oxidase, succinate oxidase and the complete respiratory chain from NADH: cytochrome *c* oxidoreductase, succinate: coenzyme Q oxidoreductase, cytochrome oxidase and cytochrome *c* was studied under various conditions. The formation of these multi-enzyme systems was prevented by cobra venom phospholipase. Reconstitution was possible in the presence of cobra venom only if the medium contained NADH (or succinate) and O_2. Bovine serum albumin prevented the formation of NADH oxidase at low temperatures but hardly affected this process at 38–42°. It also increased the thermal stability of the reconstituted system. Reconstitution of NADH oxidase did not occur in the presence of potassium oleate, and bovine serum albumin completely eliminated the effect of the latter. However, bovine serum albumin did not protect the respiratory chain from the action of phospholipase. Therefore, the presence of NADH was necessary for the reconstitution of NADH oxidase at 38° in a medium containing bovine serum albumin and cobra venom.

Thus, the natural agents indicated above have a substantial effect on the reconstitution of the respiratory chain. Reconstitution becomes possible with a strictly definite ratio between the effects of different external factors. A special part in the formation of the respiratory chain is played by substrates having a specific influence on its structure.

INTRODUCTION

The formation of the mitochondrial respiratory chain was first considered at the molecular level by KEILIN AND KING[1] who showed that succinate oxidase can be reconstituted from succinate dehydrogenase, cytochrome *c* and a heart muscle preparation freed from these components but possessing cytochrome oxidase activity. Somewhat later HATEFI *et al.*[2,3] reconstituted the respiratory chain and some of its fragments from simple enzyme complexes. Other papers devoted to a study of the reconstitution mechanism and the properties of a reconstituted respiratory chain are those of FOWLER AND RICHARDSON[4], KING *et al.*[5,6] and of GREEN *et al.*[7,8]. These authors effected the reconstitution under mild conditions wherein the respiratory chain and its fragments were capable of remaining unchanged for a long time. The

present paper deals with the influence of cobra venom, bovine serum albumin, potassium oleate and heating on the reconstitution of NADH oxidase, succinate oxidase and the complete respiratory chain. It is shown that reconstitution is possible under these conditions only in the presence of respiratory chain substrates.

MATERIALS AND METHODS

Enzymes

NADH:cytochrome *c* oxidoreductase was isolated by the method of HATEFI *et al.*[9] and cytochrome oxidase by that of GRIFFITHS AND WHARTON[10] with slight modifications[11]. The activity of the former, assayed as previously described[9], was 35–44 μmoles NADH oxidized per min per mg protein at 38°. The maximum activity of the latter was equal to 80–100 μmoles cytochrome *c* oxidized per min per mg protein at 38° (ref. 12). All the assays were carried out without adding a mixture of phospholipids, and therefore the activity values given are slightly lower than they should be[9,10]. Succinate:coenzyme Q oxidoreductase was isolated after ZIEGLER AND DOEG[13], and reduced coenzyme Q:cytochrome *c* oxidoreductase after HATEFI *et al.*[14]. The NADH:cytochrome *c* oxidoreductase preparations contained 1.5–1.9 nmoles of cytochrome *b* and 0.9–1.4 nmoles of cytochrome c_1 per mg protein and the cytochrome oxidase preparations, 6.0–7.0 nmoles cytochrome aa_3 per mg protein. The content of cytochromes *b* and c_1 in reduced coenzyme Q:cytochrome *c* oxidoreductase amounted to 3.3–4.0 nmoles and 1.7–1.9 nmoles/mg of protein, respectively. The details of determination of the cytochromes are similar to those described earlier[15].

Reconstitution of NADH oxidase, succinate oxidase and the complete respiratory chain

The respiratory chain was reconstituted from NADH:cytochrome *c* oxidoreductase, succinate:coenzyme Q oxidoreductase and cytochrome oxidase. Their concentrated solutions (21.0, 7.5 and 16.0 mg protein/ml respectively) were mixed in the proportion 2.56:1.0:0.6 in the presence of cytochrome *c* (0.3–1.0 mg/mg of cytochrome oxidase protein) and were diluted at 22° with 0.05 M Tris–HCl (pH 8.0) containing 0.66 M sucrose and 0.005 M histidine (Tris–histidine–sucrose) to a concentration 0.04 mg cytochrome oxidase protein/ml. The diluted solution contained cobra venom (7.5 mg/mg of cytochrome oxidase protein) and in some cases NADH (2.5 μmoles/ml) and/or succinate (100 μmoles/ml). Samples (0.1 ml each) were taken at definite time intervals. Their NADH oxidase activities were measured spectrophotometrically at 22° and their succinate oxidase activities polarographically. In the latter case, 2.0 μmoles/ml sodium amytal were added when necessary to inhibit NADH:coenzyme Q oxidoreductase[16] and thus NADH oxidase. The measurements were carried out under the conditions suggested earlier by HATEFI *et al.*[3]. The moment that the mixture of enzymes and cytochrome *c* was diluted was taken as the beginning of reconstitution.

When reconstituting the NADH oxidase system from NADH:cytochrome *c* oxidoreductase and cytochrome oxidase, these two enzymes were taken in the proportion 2.56:0.6 with respect to protein content. The dilution solution (Tris–histidine–sucrose) contained cobra venom (0.5 or 3.0 mg/mg of cytochrome oxidase protein), bovine serum albumin (5.0–6.0 mg/ml) or potassium oleate (10 μM). When

necessary 2.5 μmoles NADH/ml were added to the medium. In a number of cases the reconstitution was effected in the presence of two of the above substances (cobra venom and NADH, or potassium oleate and bovine serum albumin), or three of them (bovine serum albumin, cobra venom and NADH). After dilution 0.1-ml samples were taken at the time intervals indicated and NADH oxidase activities were assayed. The conditions of the assays were as follows: 0.02 M potassium phosphate (pH 7.0) with 1 mM EDTA as the medium, an enzyme concentration of 0.002–0.004 mg cytochrome oxidase protein per ml and temperature 20° or 38°. The NADH oxidase activity of the system reconstituted in the absence of cobra venom, bovine serum albumin and potassium oleate, was 45–60 μmoles NADH oxidized per min per mg cytochrome oxidase protein at 38°, or 7.5–10.0 μmoles NADH per mg total protein. The specific activity dropped 8–10-fold when the medium contained neither EDTA nor phosphate, and the enzyme concentration was 0.04 mg cytochrome oxidase protein per ml. The temperature of the medium during reconstitution was varied in different experiments from 0 to 46°.

The succinate oxidase activity of the system reconstituted from succinate: coenzyme Q oxidoreductase, reduced coenzyme Q:cytochrome *c* oxidoreductase, cytochrome oxidase and cytochrome *c* under conditions similar to those suggested earlier by HATEFI *et al.*[3], was 14–16 μmoles succinate oxidized per min per mg cytochrome oxidase protein at 38°.

Sedimentation experiments

When reconstituting NADH oxidase, concentrated solutions of NADH:cytochrome *c* oxidoreductase and cytochrome oxidase were mixed in the proportion 1:1 in the presence of cytochrome *c* (0.3 mg/mg cytochrome oxidase protein). The resulting mixture was diluted at 20° with 0.05 M Tris–HCl (pH 7.4) containing 0.25 M sucrose (Tris–sucrose), and cobra venom in an amount of 3.0–4.0 mg/mg cytochrome oxidase protein. The final enzyme concentration was 0.2 mg/ml with respect to total protein. In some of the tests the Tris–sucrose contained 2.5 μmoles NADH/ml besides cobra venom, and was saturated when necessary with N_2. After dilution and an 8-min incubation of the solution, 5 mM EDTA was added to interrupt the action of the cobra venom phospholipase[17]. The solution was then centrifuged for 30 min at 105 000 × *g*. The protein pellet was suspended in 0.5 ml of Tris–sucrose and was subjected to sucrose density-gradient centrifugation. The sucrose gradient was formed by mixing 0.5 M and 2.0 M sucrose solutions with a continuous mixing device. The samples were centrifuged for 2 h at 39 000 rev./min in a Spinco SW-39 rotor.

Succinate oxidase was reconstituted by mixing concentrated solutions of succinate:coenzyme Q oxidoreductase, reduced coenzyme Q:cytochrome *c* oxidoreductase and cytochrome oxidase in the proportion 1.0:1.56:0.6 (0.3 mg cytochrome *c* per mg cytochrome oxidase protein), and by diluting the mixture with Tris–sucrose to a concentration of 0.5 mg/ml with respect to total protein. The dilution medium contained cobra venom (3.0 or 10.0 mg/mg cytochrome oxidase protein) or cobra venom and succinate (100 μmoles/ml). 8 min after dilution, the action of the venom was interrupted by adding EDTA (5 mM), and the solution was centrifuged for 60 min at 105 000 × *g* at 0–4°. The pellet was suspended in Tris–histidine–sucrose and the suspension cleared with potassium deoxycholate, after which its difference spectrum (dithionite-reduced *minus* oxidized form) was recorded.

Reagents

The following reagents were used in this work: cytochrome *c* (Sigma, Type III), NADH (Calbiochem), bovine serum albumin (Koch-Light Laboratories) and succinic acid (Reanal). Lyophilized cobra venom was obtained from the Institute of Zoology and Parasitology of the Academy of Sciences of the Uzbek SSR (Tashkent). The phospholipase activity of the venom, estimated as the rate of splitting egg yolk phospholipids at 30°, as described in ref. 18, was not less than 1000 μequiv of free fatty acids produced per min per mg cobra venom. Oleic acid was purified before use by vacuum distillation.

Measurements were made with an EPS-3 recording spectrophotometer (Hitachi) and a PO-4 polarograph (Radiometer).

RESULTS

Reconstitution of the respiratory chain in the presence of cobra venom

The respiratory chain can be reconstituted after diluting a mixture of concentrated solutions of its fragments[3,4]. Fig. 1 shows that dilution of a mixture of NADH:cytochrome *c* oxidoreductase, succinate:coenzyme Q oxidoreductase, cytochrome oxidase and cytochrome *c* with Tris–histidine–sucrose containing cobra venom does not result in the appearance of NADH oxidase activity. However, when the medium contains NADH and succinate, in addition to the components mentioned, the NADH oxidase activity quickly reaches a value amounting to 80 % of the activity of the system reconstituted in the absence of cobra venom. The activity persists for 10 min and then falls off rapidly. Since reconstituted NADH oxidase is relatively stable at 22° (ref. 19), this inactivation is related to the action of cobra venom. Similar results were obtained on assaying the succinate oxidase activity of the reconstituted system: there was no activity in the absence of substrates.

The respiratory chain cannot be reconstituted in the presence of NADH and

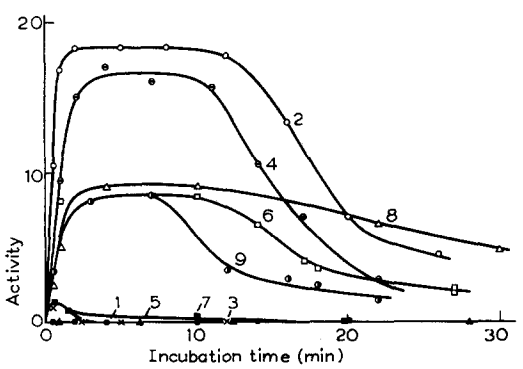
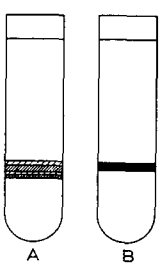


Fig. 1. Effect of substrates on reconstitution of the respiratory chain from NADH:cytochrome *c* oxidoreductase, succinate:coenzyme Q oxidoreductase, cytochrome oxidase and cytochrome *c* in the presence of cobra venom at 22°. NADH oxidase activity: Curve 1, reconstitution in the absence of NADH and succinate; 2, in the presence of NADH and succinate; 3, in the presence of NADH and succinate (anaerobic conditions); 4, in the presence of succinate. Succinate oxidase activity: Curve 5, reconstitution in the absence of NADH and succinate; 6, in the presence of NADH and succinate; 7, in the presence of NADH and succinate (anaerobic conditions); 8, in the presence of succinate; 9, in the presence of NADH. In this and in the following figures, activities are expressed as μmoles NADH or succinate oxidized per min per mg cytochrome oxidase protein.

succinate unless the dilution medium contains O_2*. Indeed, when Tris–histidine–sucrose containing cobra venom, NADH and succinate was saturated with N_2 before being added to the mixture of initial fragments, the resulting NADH oxidase and succinate oxidase activities were insignificant and disappeared soon afterwards. Succinate oxidase activity appears in the system under aerobic conditions when reconstitution occurs not only in the presence of NADH and succinate or of succinate alone, but also in the presence of NADH alone. Similarly, NADH oxidase activity appears in the presence of cobra venom when the dilution medium contains either NADH or succinate.

The reconstitution of the NADH oxidase system was studied in greater detail by sucrose density-gradient centrifugation followed by spectrophotometric and enzymic analysis of the fractions. The results of centrifugation were dependent on the conditions of dilution and incubation of the mixture of NADH:cytochrome *c* oxidoreductase, cytochrome *c* and cytochrome oxidase. When reconstitution was effected with cobra venom in the medium in the absence of NADH, the sample settled as a relatively loose layer (Fig. 2A). This layer had practically no NADH oxidase activity. It was resuspended uniformly, diluted and cleared with potassium deoxycholate and its difference spectrum (dithionite-reduced *minus* oxidized form) was recorded. An examination of the spectrum (Fig. 3A) reveals that the layer contains only insignificant amounts of cytochromes *b* and c_1 compared to cytochrome aa_3.

The situation is different when the dilution medium contains NADH. In this case sucrose density-gradient centrifugation of the sample results in a uniformly



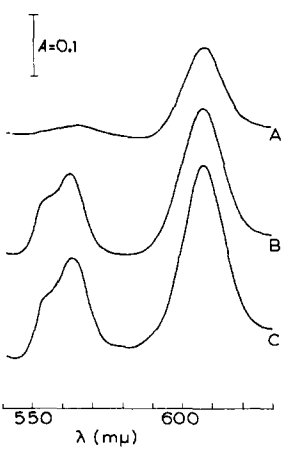


Fig. 2. Effect of NADH on the reconstitution of NADH oxidase in the presence of cobra venom at 20°. Sucrose density-gradient centrifugation data: (A) reconstitution in the absence of NADH; (B) reconstitution in the presence of NADH (aerobic conditions).

Fig. 3. Effect of NADH on reconstitution of NADH oxidase in presence of cobra venom at 20°. Difference spectra (dithionite-reduced *minus* oxidized form) of layers shown in Figs. 2A and 2B. (A) Reconstitution in the absence of NADH; (B) reconstitution in the presence of NADH (aerobic conditions); (C) spectrum of the initial mixture of NADH:cytochrome *c* oxidoreductase and cytochrome oxidase (concn. 5.0 mg of total protein per ml) without cytochrome *c*.

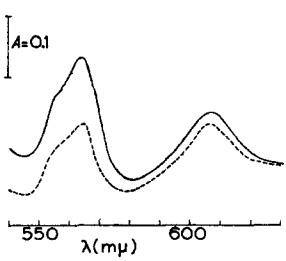
* Calculation shows that during reconstitution and subsequent incubation of NADH oxidase at 22° in Tris–histidine–sucrose containing NADH the dissolved O_2 is exhausted in approx. 7–8 min, not taking into account its diffusion from the air (see MATERIALS AND METHODS).

dense layer (Fig. 2B). Judging by the ratio of the absorption bands at 554, 563 and 605 nm, the sample contains cytochromes *b*, c_1, and aa_3 in a proportion resembling that of the initial mixture of NADH:cytochrome *c* oxidoreductase and cytochrome oxidase (Figs. 3B and 3C). The reconstituted system possesses a relatively high NADH oxidase activity, amounting to 4.5–4.8 μmoles of NADH oxidized per min per mg total protein at 38° (in the presence of 0.35 mg cytochrome *c* per ml). The activity indicated is below the true value because the layer resists resuspension. Nevertheless it is as much as 50 % of the activity of the system reconstituted in the absence of cobra venom.

It should be emphasized that no active NADH oxidase can be obtained if even one of the substrates (NADH or O_2) is lacking in the medium during the reconstitution. Indeed, when reconstitution is effected in the presence of NADH, but under anaerobic conditions, the NADH oxidase activity of the layer obtained as a result of sucrose density-gradient centrifugation is only 0.8–1.2 μmoles of NADH oxidized per min per mg total protein at 38°.

A comparison of Figs. 3A, B and C shows that cobra venom splits NADH:cytochrome *c* oxidoreductase. It is quite probable that this splitting is accompanied by detachment of reduced coenzyme Q:cytochrome *c* oxidoreductase which does not settle on centrifugation[3]. It is not impossible, however, that the splitting goes further[20,21] and that the supernatant liquid contains fragments of reduced coenzyme Q:cytochrome *c* oxidoreductase (*e.g.* cytochrome c_1 in the form of a soluble lipoprotein complex[22]) as well as some components of NADH:coenzyme Q oxidoreductase. Nevertheless, a fraction possessing high NADH oxidase activity and containing considerable amounts of cytochromes *b*, c_1 and aa_3, forms even in the presence of cobra venom if the dilution medium contains NADH and O_2 (Fig. 3B). Evidently, in the last case, the detachment of reduced coenzyme Q:cytochrome *c* oxidoreductase (or its fragments) is less noticeable.

Similar results were obtained in studying the reconstitution of succinate oxidase from succinate:coenzyme Q oxidoreductase, reduced coenzyme Q:cytochrome *c* oxidoreductase, cytochrome *c* and cytochrome oxidase in the presence of cobra venom. It follows from Fig. 4 that the reconstituted system contains larger amounts of cytochromes *b* and c_1 if the dilution medium contains succinate in addition



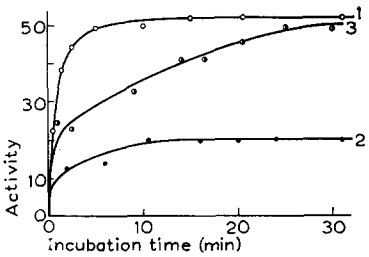


Fig. 4. Effect of succinate on reconstitution of succinate oxidase in the presence of cobra venom at 20°. Difference spectra (dithionite-reduced *minus* oxidized form). ———, reconstitution in the presence of succinate; – – – –, in the absence of succinate. Protein concentration, 7.0 mg/ml.

Fig. 5. Effect of NADH on reconstitution of NADH oxidase in the presence of bovine serum albumin at 0°. Curve 1, reconstitution in the absence of bovine serum albumin and NADH; 2, in the presence of bovine serum albumin; 3, in the presence of NADH and bovine serum albumin. Activities were measured at 38°.

to cobra venom than when succinate is absent. The amount of cytochrome aa_3 is approximately the same in both cases.

Reconstitution of NADH oxidase in the presence of bovine serum albumin

Bovine serum albumin prevents the reconstitution of NADH:cytochrome *c* oxidoreductase from NADH:coenzyme Q oxidoreductase and reduced coenzyme Q:cytochrome *c* oxidoreductase, obstructing the specific interaction of the latter[3,23]. It is evident from Fig. 5 that the reconstitution of NADH oxidase from NADH:cytochrome *c* oxidoreductase, cytochrome *c* and cytochrome oxidase at a temperature of 0–2° is also difficult in the presence of bovine serum albumin, the magnitude of its effect depending on the concentration of the latter. When the dilution medium (Tris–histidine–sucrose) contains 5.0 mg bovine serum albumin per ml, the maximum NADH oxidase activity of the reconstituted system is 35–40% of the activity attained when the reconstitution is accomplished in the absence of bovine serum albumin. However, if the medium contains bovine serum albumin and NADH, the NADH oxidase activity rises slowly and approaches its normal value. At high bovine serum albumin concentrations (10 mg/ml and more), when the reconstitution of NADH oxidase is completely depressed, the NADH effect does not occur.

The influence of bovine serum albumin on the reconstitution of NADH oxidase decreases with increasing temperature (Fig. 6). If practically no reconstitution occurs at 0°, a system forms at 38° in which the activity is almost equal to the maximum reached when reconstitution is accomplished in a medium not containing bovine serum albumin (Fig. 8). At temperatures of 38–42°, the stabilizing action of bovine serum albumin is distinctly displayed. When reconstitution occurs in its absence, the NADH oxidase activity increases to a certain value and then falls off rapidly (Fig. 8). On the other hand, in the presence of bovine serum albumin, the activity persists practically unchanged for 20 min. Further increase in the temperature reduces this effect. Thus, at 46° the maximum NADH oxidase activity of the

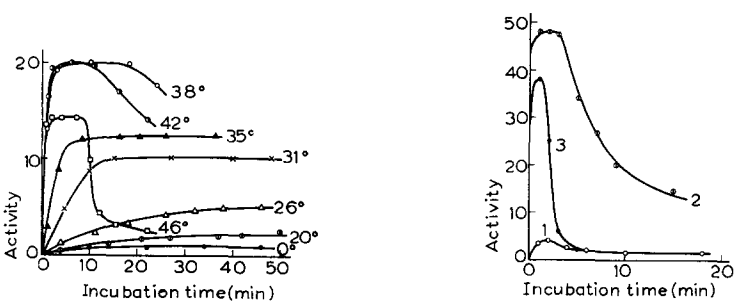


Fig. 6. Reconstitution of NADH oxidase in the presence of bovine serum albumin at different temperatures. Change in the NADH oxidase activity with time after diluting the mixture of concentrated solutions of NADH:cytochrome *c* oxidoreductase, cytochrome oxidase, and cytochrome *c* with Tris–histidine–sucrose containing bovine serum albumin. All activity measurements were made at 20°.

Fig. 7. Effect of NADH on reconstitution of NADH oxidase in the presence of cobra venom and bovine serum albumin at 38°. Curve 1, reconstitution in the presence of cobra venom (0.5 mg/mg cytochrome oxidase protein) and bovine serum albumin, without NADH; 2, in the presence of cobra venom (0.5 mg/mg cytochrome oxidase protein), bovine serum albumin and NADH; 3, in the presence of cobra venom (3.0 mg/mg of cytochrome oxidase protein), bovine serum albumin and NADH.

reconstituted system is no longer very high, and the rate of inactivation (final part of curve in Fig. 6) is considerably greater than at 42°. It should be pointed out that reconstitution proceeds best at about 38°, and that above 42° the effectiveness of this process decreases sharply.

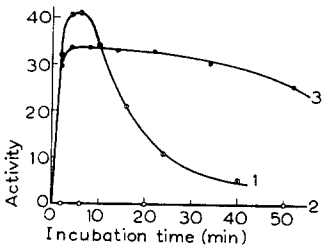


Fig. 8. Effect of bovine serum albumin on reconstitution of NADH oxidase in the presence of potassium oleate at 38°. Curve 1, reconstitution in the absence of potassium oleate and bovine serum albumin; 2, in the presence of potassium oleate (10 μM); 3, in the presence of potassium oleate and bovine serum albumin (6.0 mg/ml).

Thus, at elevated temperatures bovine serum albumin favors reconstitution of NADH oxidase, on the whole, protecting it from thermal degradation. It is important to establish whether bovine serum albumin exhibits a similar effect on the reconstitution of this multi-enzyme system in the presence of the phospholipase of cobra venom. Fig. 7 shows that NADH oxidase is practically not reconstituted from NADH: cytochrome *c* oxidoreductase, cytochrome oxidase and cytochrome *c* at 38° if the dilution medium contains bovine serum albumin and cobra venom (0.1 mg/mg total enzyme protein). However, high NADH oxidase activity appears in the presence of cobra venom, bovine serum albumin and NADH.

It follows from the above results that bovine serum albumin does not protect NADH oxidase from splitting by cobra venom. It may therefore be assumed that bovine serum albumin does not cause specific changes in the structure of enzyme particles and does not hinder access of phospholipase molecules to the latter. Experimental data also show that bovine serum albumin does not hinder the specific effect of the substrates on the reconstituted NADH oxidase consisting in enhancement of its resistance to the action of cobra venom.

Reconstitution of NADH oxidase in the presence of potassium oleate

Potassium oleate prevents reconstitution of NADH oxidase from its fragments. Fig. 8 shows the results of an experiment in which a mixture of concentrated solutions of NADH:cytochrome *c* oxidoreductase, cytochrome oxidase, and cytochrome *c* was diluted with Tris–histidine–sucrose containing potassium oleate, or bovine serum albumin and potassium oleate, at 38°. In the former case no NADH oxidase activity appeared. In the latter case, the reconstitution proceeded as in the absence of oleate. When the dilution medium contained no additives, a high NADH oxidase activity appeared which decreased rapidly, however, due to thermal degradation of NADH oxidase at 38° (ref. 11). The activity of the system reconstituted in the presence of potassium oleate and bovine serum albumin was only a little lower and persisted for some time due to the stabilizing action of bovine serum albumin (see also Fig. 6).

Thus, bovine serum albumin favors the formation of the NADH oxidase system

in the presence of potassium oleate. In contrast to bovine serum albumin, NADH does not prevent the effect of potassium oleate under the conditions indicated. It was shown earlier that free fatty acids are capable of causing profound structural changes in mitochondria, their action being eliminated by serum albumin[24-26]. The results described above are in agreement with these data.

DISCUSSION

Heart muscle preparations and electron transfer particles are inactivated when heated and are split readily by the phospholipase of cobra venom[20,27-29]. Reconstituted NADH oxidase, succinate oxidase and the complete respiratory chain are still less stable under these conditions[11,19,30]. It has also been established that the resistance of these multi-enzyme systems increases considerably when they are functioning, *i.e.* catalyzing the oxidation of NADH and/or succinate by O_2[11,19,30]. The donor, acceptor or intermediate electron carriers exhibit no protective effect if taken separately. For example, NADH does not stabilize NADH oxidase if there is no cytochrome *c* or O_2 in the medium[11,19]. Similarly, succinate does not prevent the splitting of succinate oxidase by cobra venom under anaerobic conditions[19]. It is especially interesting that the NADH oxidase activity of the respiratory chain does not decrease for some time at 38° or in the presence of cobra venom when succinate is used as the substrate, whereas its succinate oxidase activity persists under the same conditions when the substrate is NADH[30]. Succinate displays no protective effect in this case in the presence of thenoyltrifluoroacetone which inhibits succinate: coenzyme Q oxidoreductase[3]. Similarly, the effect of NADH is prevented by amytal, an inhibitor of NADH:coenzyme Q oxidoreductase[16].

The experimental data described above suggest that the formation of the respiratory chain at an elevated temperature and in the presence of phospholipase is possible only if the medium contains NADH (or succinate), cytochrome *c* and O_2. This is supported by the present study. The very fact of reconstitution of the respiratory chain under the conditions indicated signifies that in the presence of substrates it forms more rapidly than the initial fragments (*e.g.* NADH:cytochrome *c* oxidoreductase) are split by cobra venom. This becomes possible as a result of the specific effect of the substrates on the structure of the reconstituted respiratory chain. It was found recently that electron transfer particles acquire some resistance to the action of cobra venom and trypsin in the presence of NADH (or succinate) and O_2, assuming compact conformation in the functioning state[31]. This is an indication of some similarity in behavior between the intact respiratory chain and the system reconstituted from the simple enzyme complexes. The results described above are difficult to explain on the basis of the respiratory chain model suggested earlier by GREEN AND TZAGOLOFF[7,8].

Unlike the complete respiratory chain, some of its fragments (*e.g.* NADH: coenzyme Q oxidoreductase, NADH:cytochrome *c* oxidoreductase and succinate: cytochrome *c* oxidoreductase) are not protected by their substrates from thermal inactivation and from splitting by cobra venom[11,19,32]. Moreover, it has been established that NADH causes dissociation and inactivation of NADH dehydrogenase under aerobic conditions[33]. Probably only particles which catalyze the oxidation of NADH and/or succinate by O_2 are relatively stable when heated in the presence of

phospholipase and proteinase due to the features of their structural organization. Under the same conditions individual enzyme complexes and their random associates should split into smaller molecular fragments. These experimental facts suggest that, at least in model systems, the composition and structure of the respiratory chain can be controlled during its formation.

The results of this work indicate that some natural agents have a substantial effect on the reconstitution of the respiratory chain. Thus, the formation of NADH oxidase in the presence of potassium oleate is possible only if the medium contains a sufficient amount of bovine serum albumin. However, the latter hinders reconstitution at low temperatures, and in order to carry it out, the medium must be heated to 38 or 40°. On the other hand, raising the temperature results in thermal degradation of the respiratory chain and increases the rate of its splitting by phospholipase. Finally, these processes can be retarded by adding the substrates to the medium. Thus, the formation of the respiratory chain is accomplished with a strictly definite ratio between the effects of various external factors.

REFERENCES

1 D. KEILIN AND T. E. KING, *Nature*, 181 (1958) 1520.
2 Y. HATEFI, A. G. HAAVIK AND D. E. GRIFFITHS, *Biochem. Biophys. Res. Commun.*, 4 (1961) 447.
3 Y. HATEFI, A. G. HAAVIK, L. R. FOWLER AND D. E. GRIFFITHS, *J. Biol. Chem.*, 237 (1962) 2661.
4 L. R. FOWLER AND S. H. RICHARDSON, *J. Biol. Chem.*, 238 (1963) 456.
5 T. E. KING, *J. Biol. Chem.*, 238 (1963) 4037.
6 T. E. KING AND SH. TAKEMORI, *J. Biol. Chem.*, 239 (1964) 3559.
7 D. E. GREEN AND A. TZAGOLOFF, *Arch. Biochem. Biophys.*, 116 (1966) 293.
8 A. TZAGOLOFF, D. H. MACLENNAN, D. G. MCCONNELL AND D. E. GREEN, *J. Biol. Chem.*, 242 (1967) 2051.
9 Y. HATEFI , A. G. HAAVIK AND P. JURTSHUK, *Biochim. Biophys. Acta*, 52 (1961) 1850.
10 D. E. GRIFFITHS AND D. C. WHARTON, *J. Biol. Chem.*, 236 (1961) 1850.
11 V. N. LUZIKOV, M. M. RAKHIMOV AND I. V. BEREZIN, *Biokhimiya*, 32 (1967) 786.
12 D. C. WHARTON AND D. E. GRIFFITHS, *Arch. Biochem. Biophys.*, 96 (1961) 103.
13 D. M. ZIEGLER AND K. A. DOEG, *Arch. Biochem. Biophys.*, 97 (1962) 41.
14 Y. HATEFI, A. G. HAAVIK AND D. E. GRIFFITHS, *J. Biol. Chem.*, 237 (1962) 1681.
15 H. FERNANDEZ-MORAN, T. ODA, P. V. BLAIR AND D. E. GREEN, *J. Cell Biol.*, 22 (1964) 63.
16 Y. HATEFI, P. JURTSHUK AND A. G. HAAVIK, *Biochim. Biophys. Acta*, 52 (1961) 119.
17 H. M. DOERY AND J. E. PEARSON, *Biochem. J.*, 92 (1964) 599.
18 T. CREMONA AND E. B. KEARNEY, *J. Biol. Chem.*, 239 (1964) 2328.
19 V. N. LUZIKOV, M. M. RAKHIMOV, V. A. SAKS AND I. V. BEREZIN, *Biokhimiya*, 32 (1967) 1234.
20 S. MINAKAMI, F. J. SCHIENDLER AND R. W. ESTABROOK, *J. Biol. Chem.*, 239 (1964) 2042.
21 J. M. MACHINIST AND T. P. SINGER, *J. Biol. Chem.*, 240 (1965) 3182.
22 D. E. GREEN, J. JÄRNEFELT AND H. D. TISDALE, *Biochim. Biophys. Acta*, 31 (1961) 34.
23 D. E. GREEN, D. W. ALLMAN, E. BACHMAN, H. BAUM, K. KOPACZYK, E. F. KORMAN, S. LIPTON, D. H. MACLENNAN, D. G. MCCONNELL, J. F. PERDUE, J. S. RIESKE AND A. TZAGOLOFF, *Arch. Biochem. Biophys.*, 119 (1967) 312.
24 A. L. LEHNINGER AND F. L. REMMERT, *J. Biol. Chem.*, 234 (1961) 2459.
25 L. WOJTCZAK AND A. L. LEHNINGER, *Biochim. Biophys. Acta*, 51 (1961) 442.
26 A. MELLORS, A. L. TAPPEL, P. L. SAWANT AND I. D. DESAI, *Biochim. Biophys. Acta*, 143 (1967) 299.
27 R. O. MORRIS AND T. E. KING, *Biochemistry*, 1 (1962) 1017.
28 P. CERLETTI, M. G. GIOVENCO, D. BARRA AND R. STROM, *Biochim. Biophys. Acta*, 122 (1966) 352.
29 T. E. KING AND R. L. HOWARD, *Biochim. Biophys. Acta*, 59 (1962) 489.
30 V. N. LUZIKOV, M. M. RAKHIMOV AND I. V. BEREZIN, *Biokhimiya*, 33 (1968) 1115.
31 V. N. LUZIKOV, V. A. SAKS AND I. V. BEREZIN, *Biokhimiya*, 34 (1969) 874.
32 V. N. LUZIKOV, M. M. RAKHIMOV, V. A. SAKS AND I. V. BEREZIN, *Biokhimiya*, 32 (1967) 1032.
33 C. ROSSI, T. CREMONA, J. M. MACHINIST AND T. P. SINGER, *J. Biol. Chem.*, 240 (1965) 2634.