Biochimica et Biophysica Acta, 368 (1974) 29–38

BBA 46805

COOPERATIVITY IN THE INHIBITION OF OXIDATIVE PHOSPHORYLATION BY CHLOROPHENOXYISOBUTYRATE

S. R. PANINI and C. K. RAMAKRISHNA KURUP

Department of Biochemistry, Indian Institute of Science, Bangalore 560012 (India)

(Received June 12th, 1974)

SUMMARY

The kinetics of inhibition of oxidative phosphorylation by the antihypercholesterolaemic compound *p*-chlorophenoxyisobutyrate reveal cooperativity characteristic of allosteric interactions. Hill plots and Dixon plots give clear indication that the compound interferes with two distinct steps in the energy-transfer pathway. The values of interaction coefficients calculated from the Hill plots were two and four in the direction of ATP synthesis and one and two in the reverse direction. This could mean either that the pathways of synthesis and breakdown of ATP are different, or that if the pathways are the same, only half the inhibitor-binding sites function in the reverse direction.

INTRODUCTION

The ethyl ester of *p*-chlorophenoxyisobutyric acid is widely used as an antihypercholesterolaemic drug under the trade name "Atromid-S" or "Clofibrate". In addition to its hypolipidaemic action, the drug is known to cause many changes, particularly in the livers of experimental animals [1]. The compound has also been shown to affect hepatic mitochondrial content in vivo [2, 3] and function in vitro [3–6]. We have recently reported [7] that *p*-chlorophenoxyisobutyrate (CPIB) acts as an inhibitory uncoupler of oxidative phosphorylation in isolated rat liver mitochondria without any direct effect on the respiratory chain. Depending on the concentration of the compound the three sites of phosphorylation showed differential susceptibility to inhibition by CPIB.

In the course of the above investigations we observed that the pattern of inhibition of oxidative phosphorylation by CPIB showed sigmoidicity [7]. In this respect this compound resembled antimycin [8], oligomycin [9], and aurovertin [10] which also exhibit sigmoid inhibitor–effect curves. Such kinetics are characteristic of cooperative interactions which have come to be recognized as an important control

Abbreviations: CPIB, *p*-chlorophenoxyisobutyrate; TMPD, *N*, *N*, *N'*, *N'*-tetramethyl-*p*-phenylenediamine; FCCP, carbonylcyanide *p*-trifluoromethoxyphenylhydrazone; CBCNS, 5-chloro-3-*t*-butyl-2'-chloro-4'-nitrosalicylanilide.

mechanism operative in cellular processes. The role of conformational alterations in energy transduction is being increasingly appreciated in recent years [11, 12]. In accordance with this, the sigmoid inhibitor–effect curves obtained with antimycin and aurovertin have been interpreted to indicate allosteric interactions in oxidative phosphorylation [8, 10]. It was, therefore, thought worthwhile to analyze the kinetics of CPIB effects on oxidative phosphorylation and associated reactions according to the Hill equation [13–15]. The results indicate that CPIB interacts cooperatively with two functionally critical steps in the energy-transfer pathway. An abstract of the findings is being incorporated into a general discussion on allosteric interactions in oxidative phosphorylation [16].

EXPERIMENTAL

Details of the methods employed for the isolation of rat liver mitochondria as well as for the assay of oxidative phosphorylation and associated reactions have been described earlier [2, 7]. Oxidative phosphorylation was assayed manometrically. Phosphorylation coupled specifically to the first and second sites was measured using ferricyanide as the electron acceptor [17]. Energy-linked, succinate-mediated reduction of NAD^+ by reversed electron transport was determined essentially as described by Ernster and Lee [18]. The activity of ATPase (adenosine triphosphate phosphohydrolase, EC 3.6.1.3) was determined as described by Veldsema-Currie and Slater [19]. Sonic submitochondrial particles were prepared by the method of Graven et al. [20]. Other details are given in the legends to the figures.

The CPIB used in these experiments was a generous gift from Dr J. M. Thorp, Imperial Chemical Industries Ltd., U.K. The uncoupling agent carbonylcyanide-*p*-trifluoromethoxyphenylhydrazine (FCCP) and 5-chloro-3-t-butyl-2′-chloro-4′-nitrosalicylanilide (CBCNS) were gift samples from Dr W. W. Prichard, E. I. Du Pont Nemours Company, Inc., U.S.A. and Dr P. C. Hamm, Monsanto Company, U.S.A., respectively.

RESULTS

Theory

The effect of increasing concentrations of CPIB on the rates of electron transport and coupled phosphorylation was studied using tightly coupled rat liver mitochondria. As mentioned earlier, the inhibitor-effect curves (phosphorylation) were sigmoidal irrespective of the number of coupling sites activated. The Dixon Plots [21], (in which the reciprocal of the velocity of the reaction is plotted against inhibitor concentration) for the inhibition of phosphorylation were non-linear irrespective of the substrate used. (Fig. 1 insets). Such non-linearity is indicative of the interaction of more than one inhibitor molecule with the enzyme [22].

While with purified enzymes meaningful and penetrating interpretation of sigmoid kinetics is possible, the question arises of how such kinetics can be interpreted in intricate, multi-stepped processes. That complex biological phenomena such as cell division, cellular respiration etc., which show cooperative interactions, are amenable to kinetic analysis has been demonstrated by Loftfield and Eigner [15]. It was suggested that the Hill equation [13] could also be applied to such phenomena.

When applied to an enzyme system under the influence of an inhibitor, the Hill equation takes the form [14]

$$\log\left(\frac{V_0-V}{V-V_{sat}}\right) = n\log[I]+\log k$$

where V_0 is the reaction velocity in the absence of the inhibitor and V and V_{sat} are the velocities in the presence of suboptimal ($[I]$) and saturating concentrations of the inhibitor, respectively. When log $((V_0-V)/(V-V_{sat}))$ is plotted against log $[I]$ (Hill plot), the slope of the line, which has been termed the "interaction coefficient" (λ), represents a combination of the number of inhibitor-binding sites and the strength of interaction between them [14, 15, 23]. A linear Hill-plot would indicate that the inhibitor interacts with a "single critical step" in the process. On the other hand, if two or more steps with different association constants for the inhibitor are affected, it is to be expected that the slope of the Hill-plot would change with increasing concentration of the inhibitor. In other words, the number of breaks in the Hill-plot would indicate the number of critical steps being affected by the inhibitor, and the slope of each segment would indicate the minimum number of inhibitor molecules involved in the inhibition. Our analysis and interpretation of the kinetics of CPIB effects on oxidative phosphorylation and associated reactions are based on the above premises.

Rates of electron transport and coupled phosphorylation

The Hill plots for the inhibition of oxidation and coupled phosphorylation by CIPB with various substrates are given in Fig. 1. With all the three substrates, the plots for the inhibition of phosphorylation showed a break indicating that CPIB inhibits two critical steps in the energy-transfer pathway in a cooperative manner. It is interesting to note that irrespective of the number of coupling sites sites activated, the interaction coefficients always showed a value of approximately two and four for the two steps. That at least two steps in the phosphorylation pathway are sensitive to inhibition by the compound has been inferred by us earlier, based on the ability of CPIB to act both as an uncoupler (stimulation of State 4 oxidation and latent ATPase activity, abolition of respiratory control and decrease of P/O and P/$2e^-$ ratios) and as an inhibitor of phosphorylation (inhibition of State 3 oxidation, reversal of this inhibition at lower concentrations of CPIB by uncouplers and inhibition of uncoupler -stimulated ATPase activity and the ATPase activity in submitochondrial sonic particles) [7].

The inhibition of phosphorylation with ascorbate and N, N, N', N'-tetramethyl-p-phenylenediamine (TMPD) as substrate did not cause any significant inhibition of O_2 uptake (Fig. 1C inset). In this respect this compound resembled oligomycin [25, 26]. When succinate was the electron donor, the oxidation was inhibited with two interaction coefficients the values approximating to one and two respectively (Fig. 1B). In contrast to this, oxidation of glutamate+malate was inhibited yielding a single interaction coefficient approximating to two (Fig. 1A). Irrespective of the substrate used, the Dixon plots for the inhibition of phosphorylation were non-linear (Fig. 1 insets). The shape of the Dixon plot for the inhibition of oxidation appeared to be dependenton the substrate used. Glutamate and malate, which

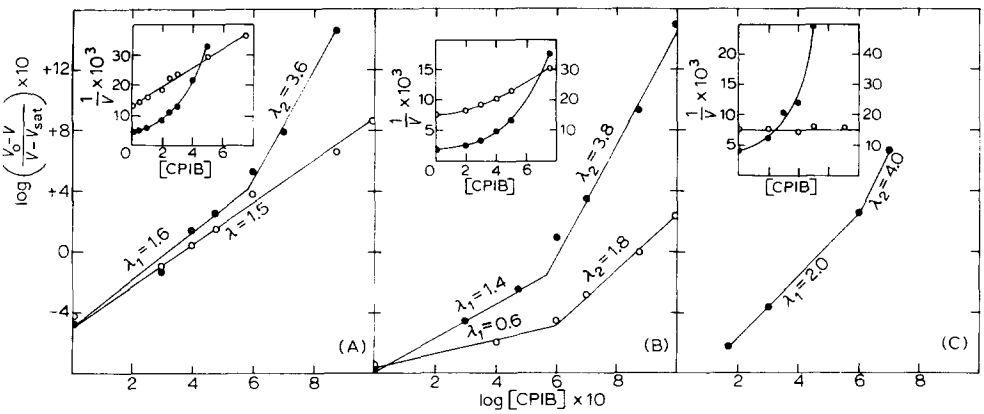


Fig. 1. Hill plots for the inhibition of oxidative phosphorylation by CPIB in rat liver mitochondria. The rates of oxidation and phosphorylation were measured manometrically [7]. The main compartment of the Warburg flask contained 150 μmoles of Tris–HCl buffer, pH 7.4; 30 μmoles of potassium phosphate buffer, pH 7.4; 6 μmoles of $MgSO_4$; 3 μmoles of EDTA; 50 μmoles of glucose; 5 μmoles of ATP; 3 mg of hexokinase (Sigma, Type III) and 4–6 mg of mitochondrial protein. The reaction volume was adjusted to 3 ml with 0.25 M sucrose. The centre well contained 0.2 ml of 20 % (w/v) KOH. After equilibration for 15 min at 30 °C the reaction was started by tipping in from a side arm 50 μmoles of glutamate+50 μmoles of malate, 100 μmoles of succinate or 100 μmoles of ascorbate+ 750 nmoles of TMPD. After measurement of O_2 uptake at 5 min intervals for 20 min (the rate of O_2 uptake was linear during this period), the reaction was stopped by the addition of 2 ml of 10 % (w/v) trichloroacetic acid. Orthophosphate remaining in the supernatant fraction was determined colorimetrically [24]. The addition of CPIB was always made from the side arm along with the substrate. When either succinate or ascorbate was used as the electron donor, 5 μg of rotenone was also added to the reaction system. The Hill plots for the inhibition of O_2 uptake (○) and phosphorylation (●) with glutamate+malate (A), succinate (B) and ascorbate+TMPD (C) are given. The inhibitor concentration is expressed as μmoles of CPIB/mg protein. With ascorbate+TMPD as the substrate phosphorylation was completely abolished above a CPIB concentration of 5 μmoles/mg protein with the result that Fig. C (●) has no points beyond this concentration. The corresponding Dixon plots are shown as insets. The values for both Hill and Dixon plots represent the mean of five independent determinations (mitochondrial preparations). The average uninhibited rates of O_2 uptake (ngatoms of oxygen/min per mg protein) were 77, 136 and 128 with glutamate+malate, succinate and ascorbate+TMPD respectively. The corresponding average rates of phosphorylation (nmoles of P_i/min per mg protein) were 208, 245 and 115 respectively. The standard deviation was less than 10 % of these values.

yielded a linear Hill plot, also gave rise to a linear Dixon plot, while succinate, which gave a biphasic Hill plot, resulted in a non-linear Dixon plot.

A similar pattern was observed in the inhibition of phosphorylation coupled to the reduction of ferricyanide (Fig. 2). When glutamate and malate acted as substrate the inhibition of electron transport activity (rate of reduction of ferricyanide) gave a linear Hill plot with an interaction coefficient approximating to two (Fig. 2A). The inhibition of phosphorylation, on the other hand, yielded two interaction coefficients approximating to two and four. With succinate, the Hill plots for the inhibition of both electron transport activity and phosphorylation showed a change in slope (Fig. 2B). As in the case of succinate oxidation (Fig. 1B), the interaction coefficients for the inhibition of electron transport activity were half those for the inhibition of phosphorylation. These trends are also reflected in the Dixon plots (Fig. 2 insets).

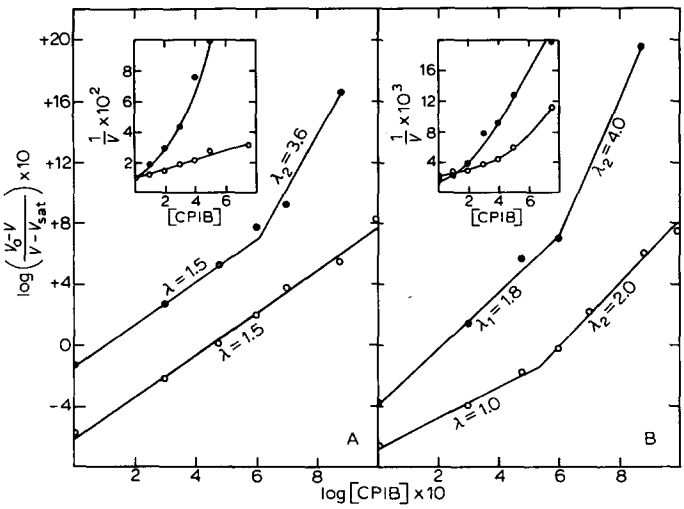
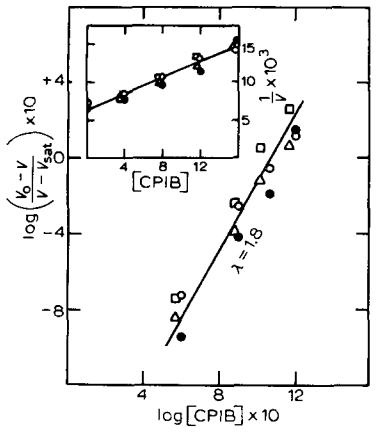


Fig. 2. Hill plots for the inhibition of phosphorylation coupled to ferricyanide reduction by CPIB in rat liver mitochondria. The reduction of ferricyanide was followed spectrophotometrically [7, 17]. The reaction system contained 50 μmoles of Tris–HCl buffer, pH 7.4; 10 μmoles of potassium phosphate buffer, pH 7.4; 1.5 μmoles of $MgSO_4$; 3 μmoles of KCN; 1 μmole of EDTA; 50 μmoles of glucose; 2 μmoles of ATP; 1.5 mg of hexokinase (Sigma, Type III); 10 μmoles of succinate or 5 μmoles of glutamate+5μ moles of malate, and 2 mg of mitochondrial protein. When succinate was the electron donor, the reaction system also contained 10 μg of rotenone. The total reaction volume was 1 ml. After preincubation at 30 °C for 5 min, the reaction was started by the addition of 10 μmoles of $K_3Fe(CN)_6$ and allowed to proceed for 10 min, when the reaction was stopped by the addition of 1 ml of 10 % (w/v) $HClO_4$. After centrifugation, the ferricyanide remaining was determined by measuring the absorbance at 420 nm in a Beckman DU-2 spectrophotometer. The P_i remaining was estimated colorimetrically [24]. Addition of CPIB was made before preincubation. The Hill plots for the inhibition of ferricyanide-reduction (○) and phosphorylation (●) with glutamate+malate (A) and succinate (B) are given. The inhibitor concentration is expressed as μmoles of CIPB/mg protein. The Dixon plots are shown as insets. The values for both Hill and Dixon plots are the averages of four independent determinations (mitochondrial preparations). The average uninhibited rates of ferricyanide reduction (nmoles/min per mg protein) were 99 and 441 with glutamate+malate and succinate respectively. The corresponding rates of phosphorylation (nmoles P_i/min per mg protein) were 95 and 124 respectively. The standard deviation was less than 10 % of these values.

Uncoupler stimulated respiration

The compound inhibited mitochondrial respiration even in the presence of uncouplers [7]. The Hill plots and Dixon plots for this inhibition are presented in Fig. 3. The change in the slope of the Hill plots in Figs 1 and 2 occurred at a CPIB concentration of about 4 μmoles/mg mitochondrial protein. In the presence of uncouplers, significant inhibition of succinate oxidation could be observed only above this concentration. It is interesting to note that the Hill plot for the inhibition of uncoupled oxidation of succinate (Fig. 3) shows a single interaction coefficient approximating to two, which is the same as the value for the inhibition of the oxidation of succinate in tightly coupled mitochondria. The non-linearity in the Dixon plots with tightly coupled mitochondria is abolished in the presence of the uncouplers (Fig. 3 inset). It is pertinent to point out here that inhibition of oxidation in the presence of uncouplers should not be considered to indicate a direct inhibitory action on the



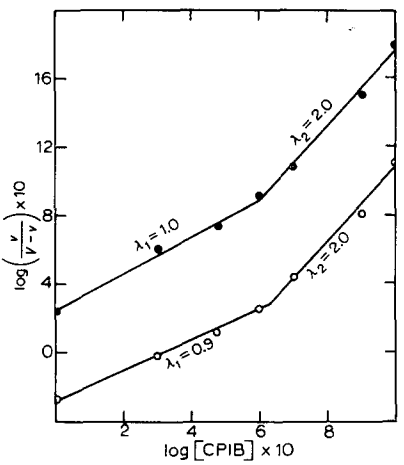


Fig. 3. Hill plot for the inhibition of uncoupler-stimulated oxidation of succinate by CPIB in rat liver mitochondria. The O_2 uptake was measured manometrically as described in the legend to Fig. 1. The reaction system did not contain glucose, ATP and hexokinase. Respiration was measured in the presence of 210 nmoles of dinitrophenol (□), 30 nmoles of dicoumarol (○), 3 nmoles of FCCP (△) or 0.3 nmole of CBCNS (●). The Dixon plot is shown as the inset. Each point in both Hill and Dixon plots represents the average of four separate experiments (mitochondrial preparations). The inhibitor concentration is expressed as μmoles of CPIB/mg protein. The uncoupler concentrations used were optimum. The average uninhibited rate of O_2 uptake (ngatoms oxygen min/ per mg protein) was 140. The standard deviation was less than 10 % of the average values.

Fig. 4. Hill plots for the stimulation of latent ATPase activity by CPIB in rat liver mitochondria. The reaction medium for the assay of ATPase activity contained 50 μmoles of Tris–HCl buffer, pH 7.4; 75 μmoles of KCl and 1 μmole of EDTA and 1.0 mg of mitochondrial protein in a final volume of 1 ml. The reaction was carried out in the presence (●) or the absence (○) of 5 μmoles of $MgCl_2$. After preincubation for 5 min, the reaction was started by the addition of 6 μmoles of ATP and allowed to proceed for 10 min, when the reaction was terminated by the addition of 1 ml of 10 % (w/v) trichloroacetic acid. After centrifugation, the P_i released was determined colorimetrically [24]. The Hill plots are constructed according to the equation

$$\log\left(\frac{v}{V-v}\right) = n \log[M] - \log k$$

where v is the velocity in the presence of the positive modifier ([M]) and V is the maximally stimulated activity [14]. Thus $\log(v/V-v)$ is plotted against logCPIB. The concentration of CPIB is expressed in μmoles/mg protein. The values are the mean of four independent determinations (mitochondrial preparations). The average latent ATPase activities (nmoles P_i released/min per mg protein) were 25 and 13 in the presence and the absence of added Mg^{2+}, respectively. The corresponding maximally stimulated activities (mean) were 66 and 68 respectively. The standard deviation was less than 10 % of the average values.

respiratory chain, since CPIB does not inhibit the oxidation of either NADH or succinate in submitochondrial sonic particles. This indicated that the compound may have two inhibiting sites in the energy-transfer pathway; one on the ATP side of the dinitrophenol site (like oligomycin) and another on the respiratory-chain side [7].

ATPase

The compound stimulated the latent ATPase activity of tightly coupled mitochondria both in the presence and absence of added Mg^{2+}. This stimulation was

totally prevented by oligomycin [7]. The Hill plots for this stimulation, constructed according to Atkinson [14], showed two interaction coefficients approximating to one and two (Fig. 4). The presence or absence of $Mg2^+$ did not affect the interaction coefficients. The occurrence of two interaction coefficients indicates that stimulation of latent ATPase activity by CPIB involves two steps.

The compound inhibited uncoupler-stimulated ATPase activity in intact mitochondria as well as in sonic submitochondrial particles [7]. The Hill plots (Figs 5 and 6) show that the inhibition in both cases proceeds in two steps with interaction coefficients approximating to one and two respectively. The Dixon plots in both cases were non-linear (Figs 5 and 6 insets).

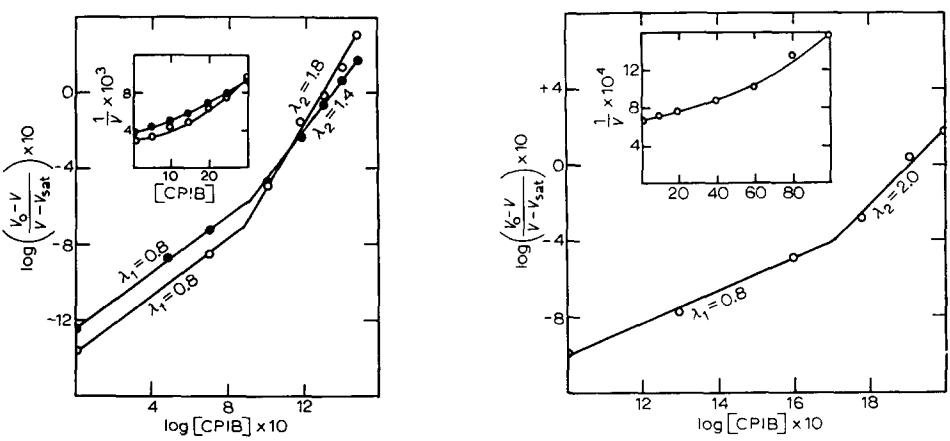


Fig. 5. Hill plots for the inhibition of uncoupler-stimulated ATPase activity by CPIB in rat liver mitochondria. The enzyme activity was assayed as described in the legend to Fig. 4 in the presence of 200 nmoles of dinitrophenol with (●) and without (○) the addition of 5 μmoles of $MgCl_2$. The mitochondrial protein was 0.5 mg and the incubation period was 5 min. The Dixon plots are shown as inset. The values for both Hill and Dixon plots are the average of five separate determinations (mitochondrial preparations). The average uninhibited activities (nmoles P_i released/min per mg protein) were 248 and 316 in the presence and the absence of added Mg^{2+}, respectively. The standard deviation was less than 15 % of the average values.

Fig. 6. Hill plot for the inhibition of ATPase activity by CPIB in submitochondrial sonic particles. The enzyme activity was assayed as described in the legend to Fig. 4 in the presence of 5 μmoles of $MgCl_2$. The particle protein was 0.2 mg and the incubation period was 5 min. The Dixon plot is shown as inset (abscissa; CPIB concentration). The values for both Hill and Dixon plots are the average of four experiments. The average uninhibited activity (nmoles P_i released/min per mg protein), was 1516. The standard deviation was less than 5 % of the average values.

Reversed electron transport

The compound effectively inhibited ATP-dependent, succinate-mediated reduction of NAD^+ in submitochondrial particles. The Hill plot in this case also showed two interaction coefficients, the values of which were one and two. The Dixon plot was non-linear (Fig. 7).

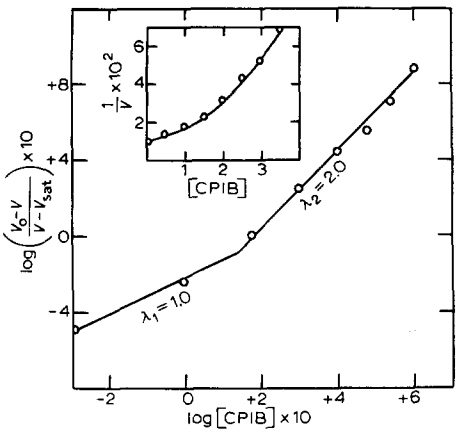


Fig. 7. Hill plot for the inhibition of ATP-dependent reduction of NAD^+ by reversed electron transport by CPIB in submitochondrial particles. The reaction system contained 150 μmoles of Tris-HCl buffer, pH 7.4; 18 μmoles of $MgSO_4$; 9 μmoles of KCN; 3 μmoles of NAD^+; 3 μmoles of EDTA; 15 μmoles of succinate; 540 μmoles of sucrose; and 1–3 mg of submitochondrial particle protein in a total volume of 3 ml. The reaction was started by the addition of 3 μmoles of ATP and the rate of reduction of NAD^+ was followed by measuring the increase in absorbance at 340 nm in a Cary 14 recording spectrophotometer. That the increase was due to NADH formation was confirmed by its reversal by the addition of pyruvate + lactate dehydrogenase at the end of the reaction. The Dixon plot is shown as inset. The values for both Hill and Dixon plots are the average of five experiments. The average uninhibited rate of reduction of NAD^+ (nmoles/min^{-1} per mg^{-1} protein) was 87. The standard deviation was less than 10 % of the average values.

DISCUSSION

As stated earlier, our analysis of the kinetics of inhibition of oxidative phosphorylation and associated reactions by CPIB, and our interpretation of the results are based on the following assumptions: (1) a break in the Hill plot signifies that another critical step in the pathway is being affected by the modifier, and (2) the slope of each segment gives the minimum number of modifier molecules involved in the modification of that step. This is somewhat at variance with the conclusions of Loftfield and Eigner [15] who proposed that as new steps are affected, the effect becomes cumulative and the apparent molecular order of inhibition therefore increases. This would presuppose that the value of the interaction coefficient can only increase with increasing concentration of the inhibitor. However, we have observed in the case of antimycin [16], that the value could actually decrease with increasing concentration of the inhibitor. Again, succinate oxidation is inhibited with an interaction coefficient of unity below a CPIB concentration of 4 μmoles/mg protein and of two above this concentration (Fig. 1B). In the presence of uncouplers the inhibition is manifest only above a CPIB concentration of 4 μmoles/mg protein and the interaction coefficient retains the value of two (Fig. 3). Were the interaction cumulative, the value should have decreased to unity. This lends support to our assumption that the slope of each segment of a Hill plot is a parameter characteristic of that step.

It is interesting to note that in all instances where the Hill plots showed a break, the Dixon plots were non-linear (Figs 1, 2, 5–7). When the break in the Hill plot was

abolished by a change in the experimental condition, the Dixon plot became linear (compare Figs 1B and 3). A linear Dixon plot always yielded a linear Hill plot (Figs 1A, 2A and 3). These results indicate that non-linearity in the Dixon plot for the inhibition of a multi-stepped process would serve as a diagnostic test to show whether more than one step in the process is being interfered with by the inhibitor. It may be mentioned that rotenone, piericidin A, oligomycin and atractyloside, which yielded linerar Hill plots [16], also yielded linear Dixon plots while with antimycin, aurovertin and bongkrekic acid both Hill [16] and Dixon plots were non-linear (Figs not given).

Another point that merits discussion is the difference observed in the Hill plots for the oxidation of malate and succinate. Since CPIB does not affect the respiratory chain directly [7], it is reasonable to presume that the inhibition of oxidation is a reflection of the inhibition of phosphorylation. While phosphorylation with both substrates was inhibited with interaction coefficients approximating to two and four (Figs 1 and 2) the Hill plot for the inhibition of malate oxidation gave a single interaction coefficient (λ = approx. 2, Figs 1A and 2A) and the plot for the inhibition of succinate oxidation gives two interaction coefficients approximating to one and two (Figs 1B and 2B). The higher value of interaction coefficient for malate oxidation may be a reflection of the tighter coupling generally observed with NAD^+-linked substrates. Because of this tight coupling the order of inhibition of oxidation may not change with a change in the order of inhibition of phosphorylation, In the case of succinate, on the other hand, the coupling being less tight ($\lambda = 1$) an increase in the order of inhibition of phosphorylation results in a change in the order of inhibition of oxidation as well. Another reason for this difference may be a possible interference of CPIB with malate dehydrogenase which was not tested. The compound, however, does not inhibit succinate dehydrogenase [7].

The results presented here indicate that the energy-transfer pathway is inhibited by CPIB at two critical steps whether measured in the forward direction (Figs 1 and 2) or in the reverse direction (Figs 4–7). It is intriguing that while the interaction coefficients in the direction of ATP synthesis are two and four, the values in the reverse direction are half of these. This could mean either that the pathways of synthesis and breakdown of ATP are different as suggested by Lardy et al. [27] or that if the pathways are the same, only half the inhibitor-binding sites function in the reverse direction. It may be pointed out that of the two binding sites on ATPase for aurovertin only one is functional [28].

The stimulation of latent ATPase by CPIB appears to proceed in two steps (Fig. 4). In this respect the compound resembles the classical uncoupler dinitrophenol which has also been found to simulate this activity in two steps with interaction coefficients of one and two [16].

The significance of allosteric interactions in membrane function has been emphasized by Changeux et al. [29, 30]. The possibility of such interactions in oxidative phosphorylation has been indicated by Slater [11]. who based his interpretation on the model of Monod et al. [31]. We have shown in this paper that the application of Hill treatment to the kinetics of inhibition of mitochondrial reactions by CPIB also indicates the play of cooperative forces in mitochondrial energy transduction.

ACKNOWLEDGEMENTS

We wish to thank Professor T. Ramasarma and Dr V. Sitaramam for the many helpful discussions we had with them. S.R.P. is a scholar of the National Council of Educational Reserach and Training, India.

REFERENCES

1 Steinberg, D. (1969) in Atherosclerosis (Jones, R. J., ed.), pp. 500–508, Springer-Verlag, New York
2 Kurup, C. K. R., Aithal, H. N. and Ramasarma, T. (1970) Biochem. J. 116, 773–779
3 Krishnakantha, T. P. and Kurup, C. K. R. (1972) Biochem. J. 130, 167–175
4 Katyal, S. L., Saha, J. and Kabara, J. J. (1972) Biochem. Pharmacol. 21, 747–751
5 Yeh, R. and Kabara, J. J. (1972) Life Sci. 11, 709–716
6 Mackerer, C. R., Haettinger, J. R. and Hutsell, T. C. (1973) Biochem. Pharmacol. 22, 513–519
7 Panini, S. R. and Kurup, C. K. R. (1974) Biochem. J., in the press
8 Bryla, J., Kaniuga, Z. and Slater, E. C. (1969) Biochim. Biophys. Acta 189, 317–326
9 Slater, E. C. and ter Welle, H. F. (1969) in Inhibitors-Tools in Cell Research (Bücher, Th. and Sies, H., eds), pp. 258–278, Springer-Verlag, Berlin
10 Bertina, R. M., Schrier, P. I. and Slater, E. C. (1973) Biochim. Biophys. Acta 305, 503–518
11 Slater, E. C. (1969) in Mitochondria – Structure and Function (Ernster, L. and Drahota, Z., eds), pp. 205–217, Academic Press, London
12 Green, D. E. and Ji, S. (1972) Bioenergetics 3, 159–202
13 Hill, A. V. (1913) Biochem. J. 7, 471–480
14 Atkinson, D. E. (1966) Annu. Rev. Biochem. 35, 85–124
15 Loftfield, R. B. and Eigner, E. A. (1969) Science 164, 305–308
16 Panini, S. R. and Kurup, C. K. R. (1974) in Proceedings of International Symposium on Biomembranes (Packer, L., ed.), Madurai, 1973, Academic Press, London, in the press
17 Lee, C. P., Sottocasa, G. L. and Ernster, L. (1967) Methods Enzymol. 10, 33–37
18 Ernster, L. and Lee, C. P. (1967) Methods Enzymol. 10, 729–738
19 Veldsema-Currie, R. D. and Slater, E. C. (1968) Biochim. Biophys. Acta 162, 310–319
20 Graven, S. N., Lardy, H. A. and Estrada-O, S. (1967) Biochemistry 6, 365–371
21 Dixon, M. and Webb, E. C. (1965) Enzymes, pp. 328–330, Academic Press, London
22 Webb, J. L. (1963) Enzyme and Metablic Inhibitors, Vol. 1, p. 174, Academic Press, New York
23 Koshland, Jr, D. E. (1970) The Enzymes (Boyer, P. D., ed.), Vol. 1, pp. 341–396, Academic Press, New York
24 Fiske, C. H. and SubbaRow, Y. (1925) J. Biol. Chem. 66, 375–400
25 Huijing, F. and Slater, E. C. (1961) J. Biochem. (Tokyo) 49, 493–501
26 Guillory, R. J. and Slater, E. C. (1965) Biochim. Biophys. Acta 105, 221–232
27 Lardy, H. A., Connelly, J. L. and Johnson, D. (1964) Biochemistry 3, 1961–1968
28 Chang, T. M. and Penefsky, H. S. (1973) J. Biol. Chem. 248, 2746–2754
29 Changeux, J. P., Thiery, J., Tung ,Y. and Kittel, C. (1967) Proc. Natl. Acad. Sci. U.S. 57, 335–341
30 Changeux, J. P. and Thiery, J. (1968) Regulatory Functions of Biological Membranes (Jarnefelt, J., ed.), pp. 116–138, Elsevier Publishing Co., Amsterdam
31 Monod, J., Wyman, J. and Changeux, J. P. (1965) J. Mol. Biol. 12, 88–118