O_2-consumption was detected by an YSI oxygen monitor at 25 °C in a reaction mixture containing in a final volume of 3.10 ml: 10 μmoles of $MgCl_2$, 2.25 mg of proteins and 310 μmoles of potassium phosphate at pH 7.40. Reaction mixtures for malate dehydrogenase and succinate dependent NAD^+ reduction were made according to Englard and Siegel[14]. Reagents were purchased from Sigma and Boehringer.

Results and discussion. The characteristics of our preparation are shown in the table. It is evident that malate dehydrogenase specific activity is very low if compared to more sophisticated preparations[11–13].

Figure 1A shows that a) our preparation oxidizes NADH and this oxidation is rotenone-sensitive; b) 500 nmoles of added succinate are stoichiometrically oxidized, but further added amounts are not. The inhibition, as figure 1B demonstrates, is due to NAD^+ generated during NADH oxidation. In fact, succinate oxidase is inhibited by NAD^+ when rotenone is added before succinate. The addition of NADH removes this inhibition, but, due to rotenone presence and malonate sensitivity (figures 1B and 2A), consequent O_2-uptake can only be attributed to succinate and not to NADH. It is likely that, in the presence of NAD^+, oxaloacetate forms from succinate, due to fumarase and malate dehydrogenase contamination (table), and this results in succinate oxidase inhibition. The addition of NADH, in the presence of rotenone, shifts oxaloacetate toward malate, as shown in figure 1C, so removing the inhibition which is repristinated by further oxaloacetate.

Successively added succinate amounts are stoichiometrically oxidized, as shown in figure 2A, but soon after NAD^+ addition O_2-consumption decreases and succinate oxidase activity becomes inhibited. If NAD^+ is added to the reaction mixture before succinate (figure 1B), it will take some time before succinate oxidase be inhibited, possibly because fumarate and malate levels must increase before oxaloacetate is produced. The preparation is antimycin-sensitive with respect to NADH and succinate oxidations (figures 2A and 2C), which is suggestive of complex I, II and III interaction. The finding that NAD^+ inhibits succinate oxidase when rotenone is present (figure 1B) and that NADH removes inhibition in the presence of rotenone (figures 1B and 1C) would rule out Davis's et al.[15] claim that 'both NADH and succinate inhibit the rate of oxidation of the other' by competing for a common respiratory assembly. This supports the suggestion that some of the interactions observed in beef heart non-phosphorylating submitochondrial particles could be artifacts due to matrix enzymes contamination. Moreover, Davis et al.[15] found that NAD^+ does not lower succinate oxidase activity, perhaps because the authors take into account only initial oxidative rates and not what occurs in the time; in fact we have shown that NAD^+ inhibits succinate oxidase only after some lapse of time (figure 2B).

Figure 2C demonstrates that if fumarate and NAD^+ are added to the reaction mixture before succinate, succinate oxidase is early inhibited. Then the quite undetectable O_2-consumption should be enough to produce oxaloacetate to such an extent as to be inhibitory for succinate oxidase. The inhibition by NAD^+ does not appear to be energy-linked, since 2,4-dinitrophenol does not remove it (figure 2C); only the addition of a large succinate amount removes inhibition, as if it were competitive, and the oxidation becomes antimycin-sensitive. Figure 2D shows that the addition of oxaloacetate at a concentration of 2.3×10^{-6} M, that is in the range of K_i for purified succinate dehydrogenase[17–20], strongly inhibits our succinate oxidase preparation, and this supports the view that the preparation is purified enough and not compartmented. On the basis of the findings here reported, and of the literature cited, we would suggest that some interactions between complex I, II and III in submitochondrial particles could also be explained by low fumarase and malate dehydrogenase contaminations. Otherwise, if the findings here reported cannot be explained on the basis of the very low oxaloacetate levels that can form in the reaction mixture, we must conclude that some inhibitory interaction may occur between complex II and NAD^+, and that this inhibition is overcome by NADH in the presence or absence of rotenone and is not energy-linked.

27 T. E. King, in: Methods in Enzymology, vol. X, p. 202. Ed. S. P. Colowick and N. O. Kaplan, Academic Press, New York 1967.

Paratopic interaction, a mechanism in the generation of structure bound enzymatic activity

H. C. Hemker and H. L. L. Frank[1]

Department of Biochemistry, Biomedical Centre, State University Limburg, NL-Maastricht (The Netherlands), 27 December 1976

Summary. A general mechanism is recognized that can cause specific enzymatic activity at interphases. It consists of 2 proteins bound in close juxtaposition at a micelle or membrane surface. One, the enzyme *sensu strictu*, bears the active site, the other, the paraenzyme, is essential for generation or specific modification of the enzymatic activity.

It is the purpose of this report to draw attention to a kind of interaction between protein molecules and an interface, that can regulate, or even generate, enzymatic activity. The basic unit of this concept consists of 2 different protein molecules adsorbed next to each other onto an interface. This configuration constitutes an enzymatically active moiety. The active site is present on one of the 2 molecules, called the active site carrier; the enzymatic activity, however, is governed by the presence of the second protein molecule, called the paraenzyme. For this kind of interaction we suggest the name

1 Present address: Department of Cardiology, St. Annadal Hospital, Maastricht, The Netherlands.

Enzymatic activities generated by paratopic interaction

Surface	Active site carrier	Para-enzyme	Substrate	Product
Phospholipid micelle	Factor X_a	Factor V_a	Prothrombin	Thrombin
Phospholipid micelle	Factor IX_a	Factor $VIII_a$	Factor X	Factor X_a
Erythrocyte surface	$C1_s$	$C1_q$ and antibody	C4 and C2	$C4_a$ and $C'2_a$
Erythrocyte surface	$C2_a$	$C4_a$	C3	$C3_a$
TUA-particles	F_1	F_o	ATP (Rutamycin-sensitive)	ADP + P_i
Phospholipid – F_4	SDH	cyt. b	Ubiquinone Oxidised (TTB–sensitive)	Ubiquinone Reduced

paratopic interaction (from the greek para – next and topos – place). It is fundamentally different from allosteric interaction[2,3] in that it occurs at an interface only and from allotopic interaction[4] in that an interaction between proteins is essential.

Materials and methods. Paratopic interaction in general is recognized by a) solubilizing the intact system[16–19], which step can be omitted in the systems existing in blood plasma as they are already in a solubilized form. Then b) separation of the constituents[6–8,11,13,16,16,20]. c) Reconstituting the system and estimating the activity as a function of the nature and amounts of components added[6,7,9,10,15–20]. For the experimental details, the reader is referred to the original literature.

Results and discussion. Perhaps the best documented of paratopic interactions is encountered in the generation of the enzyme prothrombinase, which catalyses the conversion of prothrombin (blood coagulation factor II) into thrombin. It has been suggested that prothrombinase consists of factor X_a and factor V_a (the subscript a denotes the activated factor) adsorbed next to each other onto a phospholopid surface. The evidence for this is in short: a) the minimal requirement for the generation of prothrombinase activity is the simultaneous presence of a phospholipid suspension, Ca ions and 2 proteins: the blood coagulation factor X_a and V_a[6]; b) when both proteins are bound to the same micelle, prothrombinase activity generates[7,8]; c) the kinetics for the formation of prothrombinase activity are in accordance with the model proposed[9].

In the prothrombinase complex, the active site is located in the factor X_a molecule, because a) pure factor X_a has a small but detectable prothrombinase action that can be increased 1000fold by addition of phospholipid and factor V_a. Phospholipid and factor V_a have no prothrombinase action either alone or in combination[10]; b) factor X_a is an esterase that can split synthetic esters (e.g. tosylargininemethylester) and that can be inhibited by diisopropylfluorophosphate. No enzymatic properties of factor V_a have been found[11].

Another example of paratopic interaction is the enzyme that converts factor X into its activated form via the intrinsic blood coagulation pathway. It consists of the coagulation factors IX_a and $VIII_a$ adsorbed onto a phospholipid micelle[12]. Factor IX_a is the active site here and factor $VIII_a$ is the paraenzyme. Paratopic interactions are not restricted to the blood coagulation reactions. The complement component $C1_s$ is a proesterase which, when bound to a cell surface via $C1_q$ and one IgM or 2 adjacent IgG antibody molecules, develops into an active esterase. The natural substrates of this esterase are the complement factors C4 and C2 that are converted into active forms. The latter 2 components are capable of combining and can also be adsorbed onto a cell surface. A surface bound enzyme then results that can convert still another complement factor viz. C3 into its activated form (see also the table[13–15]).

From the work of Racker and colleagues, it can be seen that paratopic interactions must also play a role in the composition of the enzyme system, catalyzing oxidative phosphorylation. In this work it is shown that among others the protein fraction F_1 and F_0 can be prepared from the inner membrane of beefheart mitochondria. F_1 has ATPase activity, but only when combined with F_0 and a particulate fraction from the inner mitochondrial membrane called TUA particles, this ATPase becomes sensitive to rutamycin (or oligomycin) as in the intact mitochondrion[16–18].

A 6th example can be found in the mitochondrion. Isolated succinate dehydrogenase (SDH) only accepts ubiquinone as a substrate and is only sensitive to inhibition by 4.4.4-trifluoro-1-(2-thienyl)-1.3-butanedione (TTB) when together with cytochrome b and particles consisting of phospholipids and coupling factor VI. The mode of interaction of active site carrier and para enzyme is unknown. Covalent bonding is unlikely to play a role, as most of the complexes described readily dissociate and recombine. Quaternary constraints analogous to these postulated by Monod et al.[3] in allosteric enzymes, but in this case asymmetrical, may be brought about in the active site

2 J. Monod, J. P. Changeux and F. Jacob, J. molec. Biol. *6*, 306 (1963).
3 J. Monod, J. Wyman and J. P. Changeux, J. molec. Biol. *12*, 88 (1965).
4 E. Racker, Fedn. Proc. *26*, 1335 (1967).
5 C. Haanen, G. Morselt and J. Schoenmakers, Thromb. Diath. haemorrh. *17*, 307 (1967).
6 D. Papahadjopoulos and D. J. Hanahan, Biochim, biophys. Acta *90*, 436 (1964).
7 E. R. Cole, J. L. Koppel and J. H. Olwin, Thromb. Diath. haemorrh. *14*, 431 (1965).
8 M. J. P. Kahn, Thesis, Leiden 1970.
9 H. C. Hemker, M. P. Esnouf, P. W. Hemker, A. C. W. Swart and R. G. Macfarlane, Nature *215*, 248 (1967).
10 F. Jobin and M. P. Esnouf, Biochem. J. *102*, 666 (1967).
11 W. J. Williams and M. P. Esnouf, Biochem. J. *84*, 52 (1962).
12 H. C. Hemker and M. J. P. Kahn, Nature *215*, 1201 (1967).
13 P. J. Lachmann. Proc. R. Soc. B. *173*, 371 (1969).
14 P. J. Lachmann, in: Clinical Aspects of Immunology. Ed. P. G. H. Gell and R. R. A. Coombs. Blackwell Scientic Publ. London.
15 H. J. Müller-Eberhard, Adv. Immun. *8*, 1 (1968).
16 Y. Kagawa and E. Racker, J. biol. Chem. *241*, 2461 (1966).
17 Y. Kagawa and E. Racker, J. biol. Chem. *241*, 2467 (1966).
18 B. Bulos and E. Racker, J. biol. Chem. *243*, 3891 (1968).
19 A. Bruni and E. Racker, J. biol. Chem. *243*, 962 (1968).
20 M. P. Esnouf, in: Human Blood Coagulation. Ed. H. C. Hemker, E. A. Loeliger and J. J. Veltkamp. Leiden University Press 1969.

carrier by the paraenzyme. The adsorption may serve as a means of orientating the molecules, and in itself may modify the tertiary structure of the protein moieties and hence influence their interaction.

Alternatively, for those allotopic complexes having a proteolytic action, one can imagine that little or no interaction between paraenzyme and active site carrier takes place, but that the substrate is more favourably bound to the complex than to the single enzyme. The observation by Esnouf that the esterolytic properties of factor X_a have not been enhanced by the formation of a prothrombinase complex hints in this direction[20].

Inhibition of tryptophan uptake in Aspergillus fumigatus by tryptamine

A. R. Gupta and K. K. Rao[1,2]

Department of Microbiology, Faculty of Science, M. S. University of Baroda, Baroda 390002 (India), 25 October 1976

Summary. The tryptophan uptake was inhibited considerably in tryptamine grown cells. This inhibition was due to feed-back inhibition and not to repression.

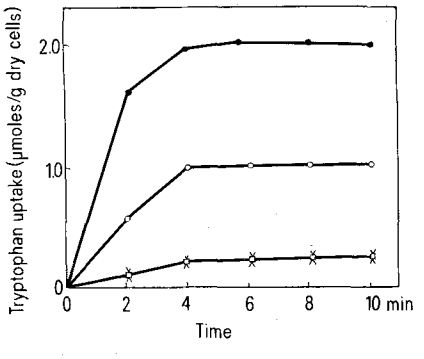
Tryptophan plays a central role in the biosynthesis of ergot alkaloids. In our previous work[3,4], we have shown that Aspergillus fumigatus mycelium can actively take up L-tryptophan from the medium and that a correleation exists between the ability of a strain to produce alkaloids and its ability to transport tryptophan. In continuation of this work on Aspergillus fumigatus, we have noticed that tryptamine inhibited the uptake of tryptophan. Wiley and Matchett[5,6] have reported the inhibition of tryptophan uptake by tryptophan and its analogues in Neurospora crassa. The purpose of this investigation was to study the mechanism of inhibition of tryptophan uptake in tryptamine grown cells.

Material and methods. A strain of A. fumigatus obtained from the Division of Mycology and Plant Pathology, IARI, New Delhi, was used in this investigation. The culture medium used was essentially that of Rao et al.[7]. Tryptamine was supplemented at the concentration of 200 mg/l. The cells were harvested after 48 h, washed with distilled water and pressed in between sheets of filter paper. Tryptophan uptake was assayed by the method of Brown and Romano[8]. The assay system contained 200 μmoles of phosphate buffer (pH 6.0), with 2.0 mg dry cells/ml in 10 ml volume; 10 μmoles of L-tryptophan were added after 30 min incubation to start the reaction. Uptake rate was calculated from initial values.

Results and discussion. We have found that tryptophan uptake was greatly reduced in tryptamine grown cells. The results presented in the table indicated that the tryptophan uptake was inhibited to the extent of 87.5% in tryptamine grown cells in comparison with ammonium citrate grown cells (considering 100% tryptophan uptake). 2 different experiments were designed to find out the mechanism of inhibition of tryptophan uptake. In one experiment, the tryptamine grown cells were washed, resuspended in ammonium citrate medium, and divided into 2 parts. Cycloheximide (50 μg/ml) was added to one culture to inhibit protein synthesis, and another culture was permitted to grow without restriction. If the cells were repressed in the presence of tryptamine and no tryptophan permease component was present, the growing cells would be expected to synthesize the transport component in the absence of tryptamine. Cycloheximide-treated cells would not be expected to synthesize this component and would remain repressed. During 48 h growth period, the cell mass of the growing culture increased significantly, indicating that protein synthesis had occurred. Tryptophan uptake studies with 48 h culture (figure) showed that there was a slight initial uptake

Inhibition of tryptophan uptake by tryptamine in Aspergillus fumigatus

Growth condition	Tryptophan uptake (μmoles/min/g dry cell)	%
Ammonium citrate	0.50	100.0
Tryptamine	0.04	12.5



Tryptophan uptake by Aspergillus fumigatus grown under various conditions. ○, Tryptamine grown cells resuspended in ammonium citrate medium. □, Tryptamine grown cells resuspended in ammonium citrate medium containing 50 μg of cycloheximide per ml. ●, Ammonium citrate grown cells (experiment 3). ×, Ammonium citrate grown cells with tryptamine added prior to experiment (experiments 1 and 2).

1 Microbiology Division, Pharmacy Department, Faculty of Technology and Engineering, M. S. University of Baroda, Baroda 390 001, India.

2 The authors are greatful to Prof. V. V. Modi for his interest in this work. The award of research fellowship by M. S. University of Baroda to A. R. G. is greatfully acknowledged.

3 K. K. Rao and V. P. Patel, Lloydia *37*, 608 (1974).
4 K. K. Rao, A. R. Gupta and V. K. Singh, Proc. Dept. of Atomic Energy (India) Symposium. Baroda 186 (1975).
5 W. R. Wiley and W. H. Matchett, J. Bacteriol. *92*, 1698 (1966).
6 W. R. Wiley and W. H. Matchett, J. Bacteriol. *95*, 951 (1968).
7 K. K. Rao, V. P. Patel and Bharti Patel, Ind. J. exp. Biol. *12*, 76 (1974).
8 C. E. Brown and A. H. Romano, J. Bacteriol. *100*, 1198 (1969).