

Paratopic interaction

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phosphorylation. In this work it is shown that among others the protein factors F₁ and F₂ can be prepared from the inner membrane of bovine mitochondria. F₁ has ATPase activity, but only when combined with F₂ and a particulate fraction from the inner mitochondrial membrane called TUA particles, the ATPase becomes sensitive to rotenone (or oligomycin) as in the intact mitochondria.¹⁴

A different example can be found in the mitochondrial isolated succinate dehydrogenase (SDH) only active in phospholipid vesicles and not active in solution by F₄.¹⁵

Another example is the enzyme TTB which 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 discussed. Coupling factor VI is unlikely to play a role, as most of the complexes described readily dissociate and recombine. Generally coupling reactions to those described by Hagedorn et al.¹⁶ in skeletal enzymes, but in this case experimental may be brought about in the active site carrier by the para-enzyme. The absorption may serve as a means of orientating the molecules, and in this way modify the tertiary structure of the protein molecules and hence influence their interaction.

Alternatively, for those enzymic complexes having a proteolytic action, one can imagine that due to an interaction between para-enzyme and active site carrier takes place, but that the substrate is not favourably bound to the complex than to the single enzyme. The observation by Lamm¹⁷ that the catalytic properties of factor X_a have not been enhanced by the formation of a prothrombin complex hints in this direction.¹⁸

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

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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 paratopic interaction. In this work it is shown that among others the protein factors F₁ and F₂ can be prepared from the inner membrane of bovine mitochondria. F₁ has ATPase activity, but only when combined with F₂ and a particulate fraction from the inner mitochondrial membrane called TUA particles, the ATPase becomes sensitive to rotenone (or oligomycin) as in the intact mitochondria.¹⁴

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Another example is the enzyme TTB which 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 discussed. Coupling factor VI is unlikely to play a role, as most of the complexes described readily dissociate and recombine. Generally coupling reactions to those described by Hagedorn et al.¹⁶ in skeletal enzymes, but in this case experimental may be brought about in the active site carrier by the para-enzyme. The absorption may serve as a means of orientating the molecules, and in this way modify the tertiary structure of the protein molecules and hence influence their interaction.

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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 (Rotamycin-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 interaction⁴ in that an interaction between proteins is essential.

Materials and methods. Paratopic interaction in general is recognized by a) solubilizing the intact system¹⁶⁻¹⁹, 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 phospholipid 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_a⁶; 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 proposed⁹.

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 combination¹⁰; 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 found¹¹.

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 micelle¹². 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 table¹³⁻¹⁵).

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 F₁ and F₀ can be prepared from the inner membrane of beefheart mitochondria. F₁ has ATPase activity, but only when combined with F₀ and a particulate fraction from the inner mitochondrial membrane called TUA particles, this ATPase becomes sensitive to rutamycin (or oligomycin) as in the intact mitochondrion¹⁶⁻¹⁸.

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 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 direction²⁰.

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