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A Macromolecule as a transducer

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Biofisica. — *A Macromolecule as a transducer.* Nota (*) del Socio straniero JEFFRIES WYMAN.

RIASSUNTO. — L'analisi presentata in questo lavoro concerne uno dei componenti emoglobinici dell'emolisato della trota (componente IV). Grazie all'eccezionale effetto Bohr, questa emoglobina può funzionare come un efficiente transduttore di energia libera, utilizzando il gradiente favorevole dei protoni per pompare ossigeno nella vescica natatoria, a fronte di pressioni anche molte alte. Il sistema è istruttivo per mostrare come un enzima multifunzionale possa usare l'energia libera prodotta in una delle reazioni catalizzate dall'enzima stesso per far procedere una seconda reazione. Viene infine discusso il caso di una macromolecola che possa funzionare da transduttore di energia libera in lavoro meccanico, o viceversa.

Several years ago I called attention to the way in which a polyfunctional enzyme (perhaps allosteric or polysteric) can in principle, owing to the linkage between the substrates which it provides, act as a transducer, using the free energy derived from one reaction to drive another [1]. The same idea has also been developed recently by Terrell Hill [2]. An interesting example of the phenomenon is provided by another type of working protein, namely component IV of trout hemoglobin, which is now being actively studied in several different laboratories. This hemoglobin is characterized by an exceptionally large oxygen-proton Bohr effect. At alkaline pH the oxygen binding curve is similar to that of human hemoglobin, showing high cooperativity with a Hill coefficient $n \cong 2$. At acid pH, however, the curve moves much faster to the right and becomes biphasic, as shown schematically in Fig. 1. This phenomenon, which is also encountered in many other fish hemoglobins, is known as the Root effect (a special instance of a Bohr effect) and makes it possible for the hemoglobin to act as an efficient molecular pump for transferring oxygen from a region of low pressure in the gills to one of high pressure in the swim bladder, where the pH is strongly acid [3].

To see how this molecular mechanism operates, suppose that the reaction rates are fast enough in relation to the velocity of blood flow so that the hemoglobin comes to equilibrium with its surroundings in both gills and swim bladder. (This in fact approximates the way things are). Let $\overline{X}$ be the fractional saturation of the hemoglobin with oxygen, x the activity (partial pressure) of oxygen and y that of proton. Let curve G in Fig. 1 describe the equilibrium behavior of the hemoglobin at the alkaline pH of the gills, curve S that at the acid pH of the swim bladder. Suppose that P_G represents the state of affairs in the gills, where $pH = -\log y_G$ and $x = x_G$, and that P_S represents

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that in the swim bladder, where $pH = -\log y_S$ and $x = x_S$. Owing to equilibrium P_G and P_S will define the state of the hemoglobin as it leaves each of these regions respectively.

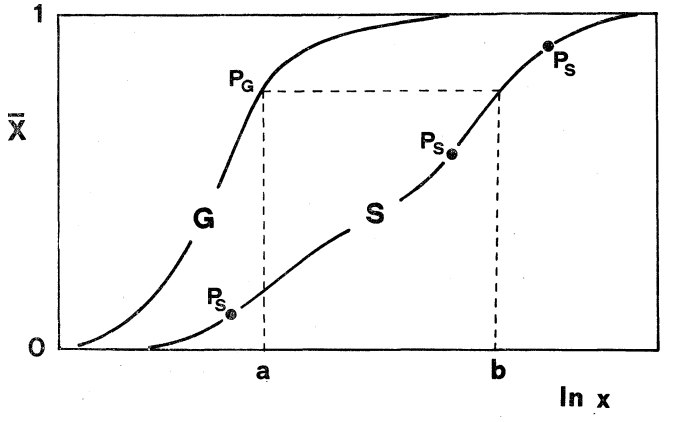


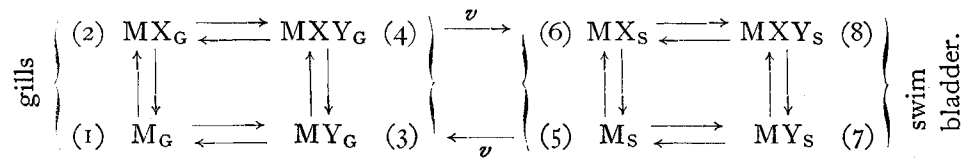
Fig. 1. - Hypothetical ligand binding curves of fish hemoglobin (e.g. Hb trout IV–Ref. [2]) at the level of the gills (G) and the swim bladder (S). For symbols see text.

Imagine now that an amount of hemoglobin is transferred from the gills to the swim bladder. There are three possibilities to consider: (i) $x_S < a$. In this case, as is apparent from Fig. 1, the hemoglobin will come to equilibrium after giving off oxygen at a partial pressure less than that in the gills, as it would have done in the absence of a Bohr effect. This case corresponds to what happens in the circulating blood in man. Here the Bohr effect simply serves to increase the amount of oxygen given off in an irreversible process. (ii) $a < x_S < b$. In this case the hemoglobin comes to equilibrium only after giving off oxygen at a partial pressure greater than that in the gills. It is under these conditions that the hemoglobin acts as a transducer, or pump, for transferring oxygen from a region of low pressure to one of higher partial pressure. (iii) $x_S > b$. In this case equilibrium is reached after an uptake of oxygen from a region of higher pressure. Of course in the special case where $x_S = b$ there will be no change in the saturation of the hemoglobin as the blood circulates—gills and swim bladder being in equilibrium as regards oxygen (but not of course proton).

Looked at as a whole the blood does not change as it circulates, although it exists in two different states, one as it leaves the capillaries of the gills, the other as it leaves those of the swim bladder. In case ii, where the hemoglobin is acting as a pump—and this is the interesting one—the work of pumping is paid for by the constant return of proton from swim bladder, where y is large, to gills, where y is smaller. The maintenance of the pH gradient which assures this continuing flow is due to the metabolism of the fish. (The thermodynamic process is of course symmetric with respect to the two ligands, oxygen and proton. Depending on the conditions, the " downhill " flow of

either one could pay for the " uphill " flow of the other). It will be seen that the whole phenomenon depends on the oxygen-proton linkage; if the curves for y_G and y_S were to coincide, the pump would fail [1].

A deeper insight into the system is provided when we write down the governing equations. In doing this, in order to prepare the way for a comparison with the enzyme system, we abandon the assumption of equilibrium in gills and swim bladder. On the other hand, we simplify the situation by substituting a simple one site molecule containing only one oxygen binding site for the more complex Hb trout IV. We also treat the system as if there were complete mixing in both sets of capillaries. By doing these two things nothing is lost in principle. We then divide the system into two parts, one for gills, one for swim bladder, according to the following scheme



Here v denotes the velocity of flow of hemoglobin (M) from gills to swim bladder and swim bladder to gills resulting from the circulation of the blood. If now we introduce the 8 velocity constants k_{12}, k_{21}, etc. for the various one step transitions, the kinetic equations may be written in the following way where the coefficients of the various forms of the macromolecule appear as a square matrix and the dot denotes time derivate.

It will be seen that the sum of the coefficients in any column of the matrix is zero, which is the conservation condition for the macromolecule. Since the x's and y's are held constant, this means that we may look for a unique steady state about which the system is asymptotically stable: this would mean that no matter what the starting point the system would always approach this unique state by a relaxation process involving in general 7 relaxation times (one less than the number of different forms (M_G, MX_G, $\cdots$, MXY_S) (1). These relaxation times would be given by the roots of the 8th degree secular equation constructed from the matrix of the coefficients (one root being zero). The relative amounts of the 8 different forms present in the steady state would be proportional to appropriate cofactors of the 8 order determinant made up of the coefficients. Depending on the values of x_G, x_S, y_G, and y_S, the transport of ligands effected by the circulation would represent one or the other of the three cases considered above. Of course, when $x_G = x_S$, $y_G = y_S$ the state of the system would degenerate into one of complete equilibrium, with

(1) So long as the pH gradient between gills and swim bladder is maintained there will of course always be a transport of proton from swim bladder to gills as the blood circulates. It is the *excess* transport resulting from the Bohr effect that meets the bill presented by the pump. This *excess* may be positive or negative depending on the position of P_S in relation to P_G. Its sign determines whether the hemoglobin acts as a proton or as an oxygen pump.

$M_G = M_S$, $MX_G = MY_S$, etc. It should be noted that the behavior of the system depends strongly on the value of v. The whole phenomenon is of course a linkage one in the general sense of the term.

EQUATION 1.

	M_G	MX_G	MY_G	MXY_G	M_S	MX_S	MY_S	MXY_S
$\dot{M}_G =$	$-k_{12}x_G$ $-k_{13}y_G$ $-v$	k_{21}	k_{31}	0	v	0	0	0
$\dot{M}X_G =$	$k_{12}x_G$	$-k_{21}$ $-k_{24}y_G$ $-v$	0	k_{42}	0	v	0	0
$\dot{M}Y_G =$	$k_{13}y_G$	0	$-k_{31}$ $-k_{34}x_G$ $-v$	k_{43}	0	0	v	0
$\dot{M}XY_G =$	0	$k_{24}y_G$	$k_{34}x_G$	$-k_{42}$ $-k_{43}$ $-v$	0	0	0	v
$\dot{M}_S =$	$+v$	0	0	0	$-k_{12}x_S$ $-k_{13}y_S$ $-v$	k_{21}	k_{31}	0
$\dot{M}X_S =$	0	v	0	0	$k_{12}x_S$	$-k_{21}$ $-k_{24}y_S$ $-v$	0	k_{42}
$\dot{M}Y_S =$	0	0	v	0	$k_{13}y_S$	0	$-k_{31}$ $-k_{34}y_S$ $-v$	k_{43}
$\dot{M}XY_S =$	0	0	0	v	0	$k_{24}y_S$	$k_{34}y_S$	$-k_{42}$ $-k_{43}$ $-v$

It is easy to see the close parallelism between this system and the complex polyfunctional enzyme operating under steady state conditions previously discussed (1). Suppose that the enzyme catalyses the reactions of two different substrates X and Y: $M + X \rightleftarrows M + X'$; $M + Y \rightleftarrows M + Y'$.

Further suppose that the catalytic efficiency of the enzyme for each substrate is dependent on the presence of the other (perhaps due to conformational i.e. allosteric effects), and, to make matters simple, assume as an extreme case that MXY is enzymatically completely inactive for both substrates The total reaction picture then becomes

$$\begin{array}{ccccc} (5)\ M + X' & \rightleftarrows & (2)\ MX & \rightleftarrows & MXY\ (4) \\ & & \uparrow\downarrow & & \uparrow\downarrow \\ & & (1)\ M & \rightleftarrows & MY\ \ (3) \rightleftarrows M + Y'\ (6) \end{array}$$

As pointed out previously (1), when the system is in a steady state, X , X′, Y , Y′ being held at arbitrary levels, we may expect a constant circulation of the macromolecule round the square, either in the clockwise or counterclockwise sense, as a result of which, under the right conditions, one of the catalytic reactions can drive the other. The only basic difference between this case and that of the hemoglobin is that in the latter the reactions are divided between two regions of space and the circulation is provided by an outside mechanical agent, the heart; in the former, everything occurs in a common solution and the circulation, which no longer represents a physical displacement in space, is itself the result of the reactions.

The kinetic equations for this system, written in the same way as Eq (1), are

EQUATION 2

	M	MX	MY	MXY
$\dot{M} =$	$-k_{12}x - k_{13}y$ $-k_{52}x' - k_{63}y'$	$k_{21} + k_{25}$	$k_{31} + k_{36}$	0
$\dot{MX} =$	$k_{12}x + k_{52}x'$	$-k_{21} - k_{25}$ $-k_{24}y$	0	k_{42}
$\dot{MY} =$	$k_{13}y + k_{63}y'$	0	$-k_{31} - k_{34}x$ $-k_{36}$	k_{43}
$\dot{MXY} =$	0	$+k_{24}y$	$k_{34}x$	$-k_{42} - k_{43}$

If we assume that the concentrations (activities) of X , X′, Y , Y′ are all held constant (as in the hemoglobin case), the coefficients of M , MX , MY, and MXY in these equations are themselves all constant and those in each column of the matrix sum to zero. Therefore, just as in the case of the hemoglobin system, we may expect the existence of a unique and asymptotically stable steady state which the system will approach, regardless of its starting point, by a relaxation process involving, in this case, 3 relaxation time. Likewise, just as in the hemoglobin case there is a constant transport of oxygen and proton between gills and swim bladder, so here there is a constant flow of X from a " reservoir " containing X at activity x to another containing X^1 at activity x^1, and similarly for Y. Either process may pay for the other.

In this simple analysis we have assumed that the activities x , x^1 , y and y^1 are maintained constant, that is to say that the system is closed with respect to the macromolecule but open with respect to the two substrates and their

end products. It is also of interest to consider the case where the system, always closed with respect to the macromolecule, is also closed with respect to one of the substrates, say X and its end product X^1, though still open with respect to the other. In this case, since the concentration of X will depend on the amount bound by the various forms of the macromolecule, namely MX and MXY, the equations are no longer linear. As a result their solution becomes vastly more complicated. It will be seen, however, that the situation here is essentially the same as that considered in a recent paper by Fichera *et al.* [4], for which it was rigorously proved that there always exists a unique critical point, asymptotically stable *in the large*, which the system will always approach, whatever its starting point, although in general it will not be possible to describe the process by any finite set of relaxation times. We may expect, therefore, the same to be true of the present system. This would mean that there is one and only one solution in terms of real positive roots of the set of equations obtained by setting all time derivatives in the kinetic equation equal to zero and invoking the conservation conditions. This solution, which may be thought of as a kind of generalized mass law, will embody the effect of the irreversible process involving Y on the " equilibrium " between X and X′ realized in the steady state.

It will be seen that in all these cases the role of the macromolecule as a transducer (a pump in the most general sense) is made possible only by the linkage between the ligands, most probably of conformational (allosteric) origin, which is provided by the macromolecule. For this reason it is of interest to broaden the linkage picture as far as possible. It has been pointed out that under conditions of equilibrium all the possible linkage relations applicable to a system (say a macromolecule and its ligands) are contained in a group of potentials derivable from the energy by a corresponding group of Legendre transformations [5]. If we extend the expression for the energy to include other forms of work than simple pressure volume work (e.g. mechanical or electrical work) the result is a corresponding extension of the group of potentials, of which the new members provide a set of new linkage relations applicable to such phenomena as thermoelastic, chemoelastic or electromechanical effects. In all such cases the additional work term will find expression in two variables, one intensive and one extensive. These will correspond to p (pressure) and V (volume) respectively. Thus, just as ΔV corresponds to ΔH or $\Delta \overline{X}$, (apart from sign) so in a thermoelastic case ΔV corresponds to Δl, where l denotes length: likewise, just as p corresponds to T or x, so in the thermoelastic case it corresponds to τ (tension). The same concept carries over to the non equilibrium case.

As an example, consider a macromolecule which is subject to a chemoelastic effect and suppose that it is exposed alternatively, with velocity (frequency) v, to two environments in one of which it is subjected to a tension τ and confronted by a ligand of activity x_1, in the other of which it is subjected to a tension τ_2 and confronted by the same ligand at activity x_2. It will be seen that the situation is essentially isomorphous with that of component IV

of trout hemoglobin with which we began the discussion. The tension length curves of the macromolecule at the two values of x are the exact equivalent of the binding curves of the hemoglobin at the two values of pH. Thus the macromolecule can act in the same way as a transducer, in this case for converting chemical free energy into mechanical work, or, conversely, for converting mechanical work into chemical free energy, depending on the conditions. This way of looking at things may be suggestive in relation to the vastly more complex situation presented by a muscle in a state of maintained contraction.

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