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Oxygen binding of arthropod hemocyanin

The role of allosteric equilibria in terms of the nested Monod-Wyman-Changeux model

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Summary. When the oxygen binding of the hemocyanin from the lobster *Homarus americanus* was analysed in terms of the nested Monod-Wyman-Changeux model, it revealed that protons affect the allosteric equilibria between four conformations. Applying computer simulations we have demonstrated the specific influence of the three different allosteric equilibrium constants on the affinity and cooperativity of oxygen binding.

Key words: Hemocyanin – Allostery – Nested MWC model

Arthropod hemocyanins are copper-containing extracellular proteins which are organized as multiples of cubic hexamers, i.e. 1×6, 2×6, 4×6, 6×6 or 8×6 (van Holde and Miller 1982; Ellerton et al. 1983). They are responsible for the oxygen transport, which can be regulated by physiological effectors like protons, inorganic ions and L-lactate. Different models have been developed to explain how allosteric effectors can change the affinity and cooperativity of ligand binding. One of these is the nested MWC model (Monod et al. 1965; Decker et al. 1986; Robert et al. 1987) which assumes hierarchies of allosteric equilibria being based on obvious structural hierarchies.

Continuous oxygen binding curves were performed for the 2×6mer hemocyanin from the lobster *Homarus americanus* at different pH values ranging over 7.0–8.5 and in the presence of physiological concentrations of Ca^{2+} and Mg^{2+} (Decker and Sterner 1990). The oxygen affinity, as well as the cooperativity, are strongly pH-dependent. The analysis of the oxygen binding curves with the classical MWC and the nested MWC model led to the following results.

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a) At particular pH values, the binding curves of *H. americanus* hemocyanin can be fitted with the classical MWC model. Comparison of the parameters obtained at different pH values, however, confirmed the results of O_2/CO competition experiments (Richey et al. 1985) that the classical MWC model cannot describe the function of the *H. americanus* satisfactorily: protons do not only influence the allosteric equilibrium constant $L=[T]/[R]$ but also the affinity constants of the two postulated conformations, k_R and k_T, and the size of the 'allosteric unit' (N).
b) In terms of the nested MWC model the half-molecules were assumed to be the allosterically coupled 'allosteric units'. Protons have no effect on the oxygen affinities of the four postulated conformations k_{rR}, k_{tR}, k_{rT} and k_{tT} but influence the allosteric equilibria between them at two different hierarchical levels.

Applying computer simulations, we changed the different allosteric equilibrium constants of the nested MWC model (L, l_R and l_T) by several orders of magnitude in order to study their influence on the affinity and cooperativity of oxygen binding (Fig. 1). The starting values for the parameters of the nested MWC model (oxygen affinity constants: k_{rR}, k_{tR}, k_{rT}, k_{tT}; allosteric equilibrium constants: L, l_R, l_T; size of the allosteric unit: N; number of the allosterically coupled allosteric units: N') are identical with the values which were determined for the *H. americanus* hemocyanin at the physiological pH of 7.66 (Mangum and Shick 1972).

The results of the simulations can be summarized as follows. Changing the values of l_R or l_T has a strong influence on the oxygen affinity, on the degree of cooperativity and on the degree of saturation where cooperativity reaches its maximum value. Variation of L has only a weak effect on the oxygen affinity, but a strong one on the degree of cooperativity. In all cases the 'cooperative range' is changed. This is the range of saturation where cooperativity occurs.

Assuming that different physiological effectors have a different influence on the three allosteric equili-

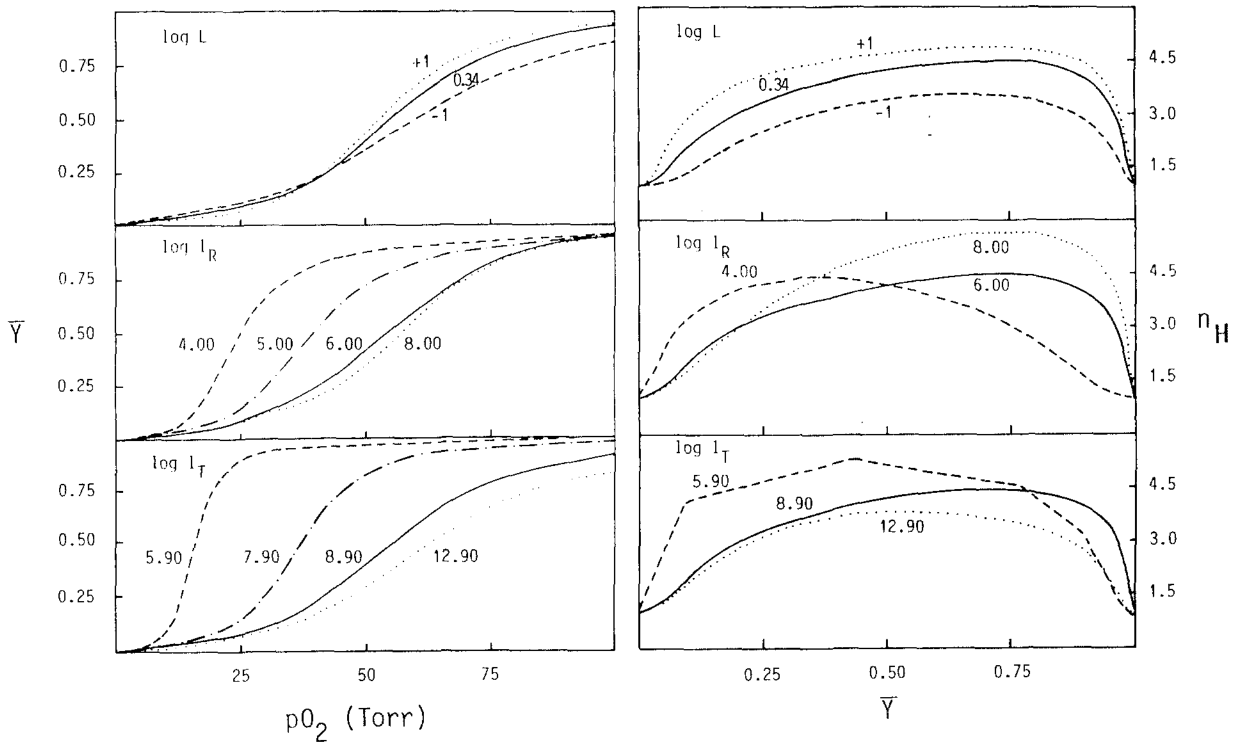

Fig. 1. The dependence of oxygen affinity and cooperativity (n_H) on the values of the allosteric equlibrium constants in the framework of the nested MWC model. The curves were simulated using the following values for the parameters: $k_{rR}=0.18$ Torr^{-1}, $k_{tR}=0.0048$ Torr^{-1}, $k_{rT}=0.61$ Torr^{-1}, $k_{tT}=0.0015$ Torr^{-1}, $\log L=0.34$, $\log l_R=6.0$, $\log l_T=8.9$, $N=6$, $N'=2$; (1 Torr $\equiv$ 133.2 Pa). These values had been calculated for the oxygen binding curves of *H. americanus* hemocyanin at the physiological pH of 7.66 (continuous lines). For the simulation all parameters except one, L or l_R or l_T, were held constant. The values for the changed allosteric equilibrium constants are given in the figure

brium constants, a very sophisticated regulation of oxygen binding would be possible. Each effector could modulate the oxygen affinity, the degree of cooperativity, the saturation at which cooperativity reaches its maximum value and the allosteric range in a specific manner. Variation of the concentrations of the different effectors according to the particular physiological situation of the animal could lead to a very efficient oxygen uptake at the respiratory surfaces and oxygen delivery at the tissues.

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