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Short communication

Influence of the solution pH on the interaction mechanisms between the molecules of the (*R*)- and (*S*)-enantiomers of a few β-receptor blocking agents and those of cellobiohydrolase I (CBH I)

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In a recent contribution, Hedeland et al. [1] reported on efforts to interpret the mechanism of the chiral separation of alprenolol on CBH I, by combining the results of microcalorimetric, enzymatic, and linear chromatographic measurements. However, neither calorimetry as used by Hedeland et al. nor linear chromatography can afford direct determinations of the binding constants of the enantiomers to the enantioselective sites on the protein. These methods measure only the global effect arising from nonselective as well as enantioselective interactions. Only methods that allow the determination of equilibrium isotherm data in a wide enough concentration range permit the separate determination of both sets of data [2]. The calorimetric measurements reported by Hedeland et al. [1] were acquired in too limited a range of experimental conditions to support the conclusions of the authors and their discussion of relevant studies dealing with the same issue [3,4]. The influence of the solution pH on the interactions between the molecules of the protein and those of β-blockers was paid no attention, except for the mention, at the end of the Experimental section [1], of the single solution pH at which the measurements were made. By contrast, the considerable influence of the solution pH on the various interaction energies involved, on the relative importance of the contributions of the enantioselective and the nonselective mechanisms in the retention of the enantiomers of propranolol, and even on the binding capacity of the protein had been amply demonstrated at the time of their work [3–5]. The same conclusions have been later extended to the enantiomers of several other β-blockers (metoprolol, alprenolol [6]).

Because of this major influence of the pH of the solution on all the thermodynamic parameters of the molecular interactions of the β-blockers studied [2–4], we fully agree with the following two comments recently made by Hedeland et al. [1]:

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(1) "(. . .) the enantiomer with the highest affinity had the highest positive enthalpy change, which was verified in a later thermodynamic study [3] based on isotherms of the two propranolol enantiomers, determined by frontal analysis (nonlinear chromatography) on a CBH-I silica column." (this is true at pH = 5.5 [3]);
(2) "in another study using the biLangmuir isotherm model, it was suggested that the observed enantioselectivity was mainly due to differences in monolayer capacities of the stereoselective sites and the (*R*)- and (*S*)-enantiomers had the same binding constants to these sites [4]." (this is true at pH = 4.7 [4]).

However, we fail to follow the rationale leading Hedeland et al. [1] to conclude that these comments are contradictory [1]. Indeed, our two studies [3,4] were carried out with solution of different pHs (5.5 and 4.7, respectively) and the different results outlined in the two statements above merely demonstrate the importance of this parameter: the chiral separation mechanism is strongly influenced by the solution pH.

In the older study [4], the equilibrium isotherms of the propranolol enantiomers were measured at pH = 4.7. At this low pH, the protein behaves as a weak chiral selector. As Hedeland et al. [1] summarized correctly (second comment above), its enantioselectivity arises from a difference in the monolayer capacities of the two enantiomers. This behavior was confirmed recently for two other β-blockers, alprenolol and metoprolol [6]. It is probably explained by the influence of the pH on the micro-environment of the enantioselective site [7].

By contrast, our more recent study [3], carried out at pH = 5.5, leads to the different conclusion, that, at this pH, the chiral separation is essentially controlled by the difference between the binding constants of the two enantiomers (see first comment above). This was illustrated in particular by a note in Table 1 [3]. Understanding the profound influence of the mobile phase pH on the thermodynamics of the molecular interactions involved, we carried out later more comprehensive investigations, in a wider pH range for propranolol [5] and, in the same wide pH range, with metoprolol and alprenolol [6]. The results regarding the true enantioselective interactions demonstrated that (1) the monolayer capacity for the *R*-enantiomers of all three compounds and (2) the true binding constant of the *S*-enantiomer increase rapidly with increasing pH [3,5,6].

Extrapolating these trends to pH = 6.8, the value at which Hedeland et al. [1] carried out their measurements, suggests that there are no actual contradictions between their results and ours. We previously reported the values of the initial slopes of the nonselective and the enantioselective isotherms of the two enantiomers of alprenolol (Table 3 in [8]). These data show that the apparent $([a_{II}(S) + a_I]/[a_{II}(R) + a_I])$ separation factor at pH = 6.02 is 4.3 (the true separation factor at pH = 6.02, $a_{II}(S)/a_{II}(R)$, is larger, at 7.4) ([8], Table 3). Hedeland et al. [1] reported a five-fold ratio for the apparent separation factor at pH = 6.8. The trends that we reported [8,9] show that the apparent separation factor increases with increasing pH between 5.01 and 6.02. This substantial agreement between our data and those reported by Hedeland et al. [1] suggests that most of the nonselective sites are carried by the protein itself.

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