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ENRICHMENT AND SEPARATION OF OPTICAL ISOMERS BY STEREOSELECTIVE ACTIVE TRANSPORT *IN VITRO*

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Abstract—A membrane containing hexokinase and phosphatase is made asymmetric by a pH gradient. D-Glucose phosphorylated in presence of ATP on entering and regenerated on leaving the membrane is consequently pumped, i.e. is actively transported through the membrane, meanwhile L-glucose diffuses passively. Enantiomer separations are made possible by using the membrane for selective pumping of the D-isomer or as a selective barrier against its retrodiffusion.

This paper deals with the concept and some demonstrations of a new technique giving the enrichment of enantiomers each side of a chiral membrane when starting with a racemic mixture. In some systems, complete or nearly complete resolution of one of the antipodes can be predicted, together with increase in concentration.

Effectively, several methods have already been proposed which are based on selective absorption (affinity chromatography)[1] or selective chemical (enzymatic) reactions followed by phase-separations of a new product[2] in column operations. Selectivities due to cyclic ether carriers[3] were also reported.

Here we are concerned with active transport membranes carrying two enzymes; the substrate to be separated entering the membrane is transformed by a first enzyme layer into an intermediate (ionic) product; in another layer, near the leaving side of membrane, the substrate is regenerated from the product by a second enzyme.[4] If the membrane is covered with external charged layers, the intermediate ionic product can be maintained inside the membrane.

We have demonstrated that in such membrane systems, the coupled inlet and outlet reactions give rise to transport phenomena and the substrate is pumped through the membrane. The concentration of the substrate becomes higher on the outlet side of the membrane than on the inlet one and the steady state concentration difference is maintained as long as the membrane is active.

In the latest model,[5,6] as in the previous ones, D-glucose was used as substrate of the first enzyme (hexokinase) and converted to glucose-6-phosphate in presence of ATP; the regeneration of glucose from its phosphate was accomplished with a second enzyme (acid-phosphatase). The differentiation of enzyme activities was created by a pH gradient through the membrane; the local pH is thus favourable to a different enzyme in two different layers of the membrane (Fig. 1). The splitting of ATP gives the necessary energy to the pumping.

Such a membrane pump can distinguish between two enantiomers, e.g. D- and L-glucose, as only one of the antipodes is a substrate. This substance will be pumped while its antipode can only passively diffuse through the membrane. This leads to two types of differences with a racemic mixture: (1) The net fluxes entering and leaving the membrane are different for the D- and L-forms before the steady state; (2) In the equilibrium state for

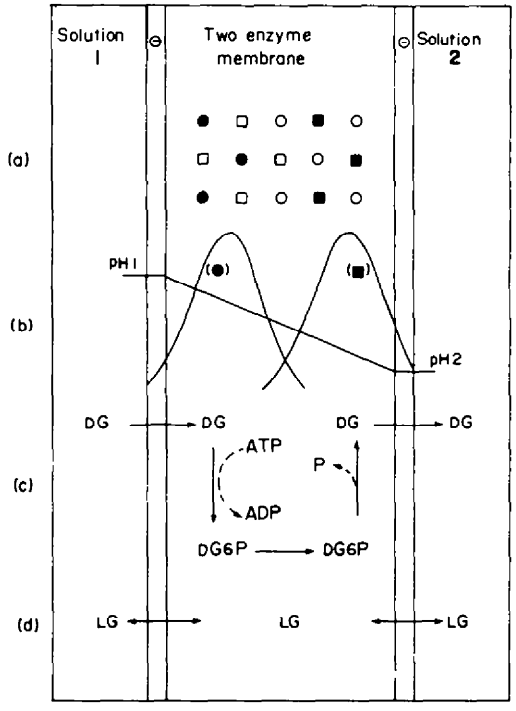

Fig. 1. (a) Statistical distribution of enzymes; (b) distribution of activities in function of local pH; (c) active diffusion reaction of D-glucose; (d) passive diffusion of L-glucose. ○ ● hexokinase; □ ■ phosphatase.

the L-antipode, its concentrations are equal on both sides of the membrane; the steady state concentration of the *substrate* is lower on the giving and higher on the receiving side, as long as the pump works.

Consequently *optical rotations of opposite sign* will appear in the two compartments limiting the membrane; e.g. if the membrane pumps from the left to the right, the optical rotation will be of the sign of the actively transported natural antipode on the right hand side. The question is to calculate the yield and the optical purity, and experimentally to demonstrate the effect; the detailed mathematical treatment[6] is given elsewhere. Let us define ΔS_1^2 as maximum concentration *difference* between the two compartments for the actively transported D-isomer (steady-state); $(S_D)_1^2$ ratio of these concen-

trations; $S_L^D = S_D/S_L$ = concentration ratios in a compartment of D- and L-forms; $OP_D = S_D/(S_D + S_L) = S_L^D/(1 + S_L^D)$ optical purity, of D-form.

Quantitative predictions can be made on the basis of the following simple properties of the pump: (1) ΔS is greater for zero order enzyme reaction ($S_D \geqslant 10\,K_M$ (Michaelis constant) (2) ΔS is smaller, but $(S_D)_1^2$ and OP_D are greater for first order reaction (e.g. $S_D \leqslant K_M/10$). (3) The numerical values of ΔS and $(S_D)_1^2$ or OP increase with the relative rate of enzyme activity (v/K_M).[5] Two major types of experimental arrangements can be made.

The membrane is used as a pump. Half-cell 1 contains a racemic mixture, membrane and half-cell 2 being free from S_D or S_L: the break-through time is the same for free diffusion of S_L and diffusion-reaction of S_D, that is both appear in the same time in half-cell 2. The ratio of D/L fluxes is constant in function of time up to the steady state where the concentration of non-natural S_L identical on both sides becomes equal to the half of the original (S_L) concentration, and ΔS_1^2 is maximum. Optical purity in half-cell 2 is constant during the whole experiment as long as the pump works; it is greater for first order kinetics (Fig. 2). OP decreases when the pump slows down.

When starting with the same *total* S_D and S_L as above but distributed as identical racemic concentrations on both sides of the membrane, the concentration of D_L will remain unchanged. During the establishment of the steady state, the optical purity (OP) will be lower on both sides than for the preceding case. However in the steady state ΔS_1^2, $(S_D)_1^2$ and OP become identical to those in the preceding case. If both half-cells are of the same volume the OP of the D form on the left side and of the L-form on the right side will be identical throughout the experiment.

The membrane is used as a selective barrier. If the starting racemic concentration is higher in the "receiving" half-cell 2 the transfer of non natural S_L from half-cell 2 to half-cell 1 will be greater than that of the *actively retained* S_D. The steady state (ΔS_1^2) will be the same as for case A; e.g. if the starting $(S_D)_2 - (S_D)_1 = (\Delta S_1^2)_{max}$ only S_L will diffuse back from 2 to 1.

It is clear that OP increases with decreasing S_1 concentration. There exists a limiting value dependent on enzyme activity for which in case A or B *all* S_D *is confined in the steady state to the receiving half-cell* 2. In this case *optically pure* S_L *is obtained on side* 1. To obtain OP = 1 for S_D, half-cell 1 must be washed by water in order to progressively eliminate S_L retrodiffusing from half-cell 2.

The method is of theoretical importance. It shows that chemical selectivity can lead simultaneously to a physical separation of enantiomers. Unless enzyme activity is very high, the method is limited to low concentrations, that is the physiological range, when high OP is desired. But consecutive membranes can be associated. The second enzyme can be replaced by a spontaneous reaction. In further extensions enzyme reactions can be replaced by other stereoselective ones.

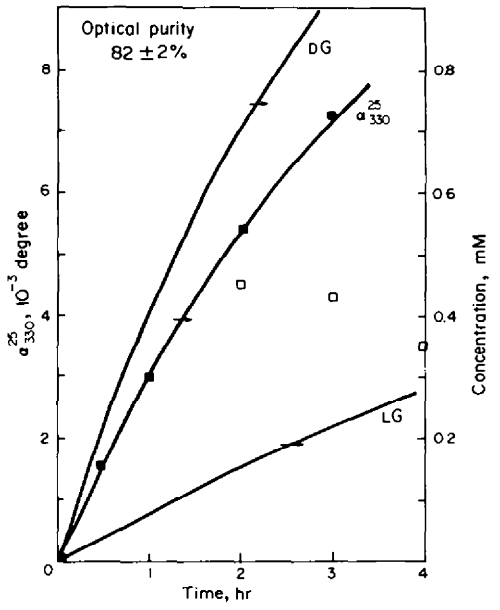

Fig. 2. Variation of optical activity (left ordinate axis) and of concentrations of L- and D-glucose (right ordinate axis) in function of time in the receiving compartment (half-cell 2). Pump: hexokinase (E. C. 2711) 1 mg, phosphatase (E. C. 3. 1. 3. 2.) 1 mg; in crosslinked agarose membrane covered by a polyacrylic acid film; pH 9,5 on side 1 and 7 on side 2. Volumes of each half-cell: 20 ml; membrane surface 1 cm². Initial concentration: 5 mM on side 1; side 2 no glucose. *Curves*: L G: passive diffusion of L-glucose and D G: active transport of D-glucose in separate experiments. α^{25}_{350} line calculated and (■) experimental optical rotations when starting with DL racemic mixture. Optical purity throughout the experiment: 82 ± 2% is typical of membrane and system; (□) optical rotations when the pump-activity is decreased by subtraction of ATP.

The selective conversions of molecules into ions can give in the synthetic field other separation techniques that will be detailed in another paper.

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