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Sensitivity increase in molecular recognition by decrease of the sensing particle size and by increase of the receptor binding site – a case with chemomechanical polymers

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For the first time it is shown that diminishing the particle size of a chemomechanical polymer leads to a dramatic sensitivity increase, with a large response triggered *e.g.* by action of external AMP; as illustrated with carbohydrate model complexes in solution, the necessary high binding affinity can be achieved by providing an excess of recognition units in one of the partners.

High sensitivity for the detection of external chemical signals is a major issue in most applications of supramolecular chemistry, and of fundamental importance in living systems.[1] We want to demonstrate until now largely overlooked ways of sensitivity increase in molecular recognition based on compartmentalization of the receptor site in combination with high capacity in terms of many available binding sites on the receptor surface. In combination with related sensitivity increases observed with receptor models for carbohydrates the results illustrate how artificial and biological complexes can recognize effector molecules in high dilution, beyond the affinity increase reached by the traditional chelate or multivalency effect which is based on additive simultaneous interactions between several binding sites.[2]

Recently we have implemented supramolecular binding sites into hydrogels, leading to selective chemically induced macroscopic expansion of such chemomechanical polymers.[3] In our earlier experiments the observed sensitivity was relatively low, due not only to relatively small affinities of the effectors, but as found now due also to other factors, evident from new measurements with smaller polymer particles with high binding capacities. Absorption of AMP in a polymethyl methacrylate-based polymer, which contains ethylenediamine and dodecylamine units as binding sites (see ESI supplement), leads to fully reversible expansions, which depend not only on the amount of AMP in the external aqueous solution, but also on the size of the used film particles. Fig. 1 shows the AMP concentration needed for a certain expansion (here 35% volume increase[4]) as a function of the polymer particle dimension, given here in variable length of the pieces. The same 35% expansion occurs at 100 mM AMP concentration using the largest polymer piece, and with only 5 mM AMP using the smallest particle, which with 1.2 × 5 × 0.4 mm is far from a possible miniaturization. The question then is, how far one can expect a further sensitivity increase with downsizing the receptor compartment.

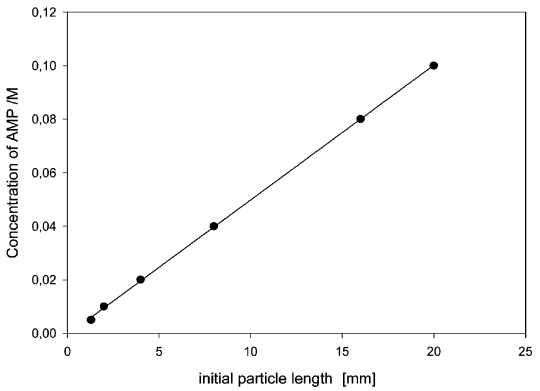

Fig. 1 Effector AMP concentration needed for a certain expansion (here 35% in three dimensions) as a function of the polymer particle size (variable length, with constant width and thickness; in 0.02 M phosphate buffer, pH = 7.0).

The situation is illustrated with Fig. 2, where either larger or smaller compartments with receptor sites on their surface bind effector molecules from the surrounding medium. If the compartment is larger one needs more effector molecules to occupy the available binding sites on the receptor (case I), than needed for a smaller unit, which therefore can operate also in more dilute solution (case II). In both cases the relative signal within the receptor compartment, such as the expansion of a chemomechanical polymer, or an effector-induced change of an optical density, will be the same, with a smaller compartment possibly also in more dilute solution. The resulting gain in sensitivity by miniaturization of the signaling unit has been described already for an optical sensor.[5] However, there are two essential prerequisites to be met for such intriguing applications: (i) the number of receptor binding sites in the unit must be large enough to bind as many effector molecules as possible out of the surrounding medium, and (ii) the affinity must be high enough to allow binding also under high dilution. This is the most demanding limitation, which calls for the design of receptors with optimal binding strength – a question addressed below.

Several findings suggest how an affinity increase can also be obtained by providing a receptor unit with significantly more binding sites than needed for the operation of a classical chelate effect. Most artificial host compounds such as ionophores are so designed that each binding site can be in optimal contact with all available binding sites of the guest (Fig. 3, cases a, b); in a chemomechanical polymer as well as *e.g.* in antibody–antigen associations, the host such as a cell surface or an antibody bears more binding sites than a single effector molecule can make use of

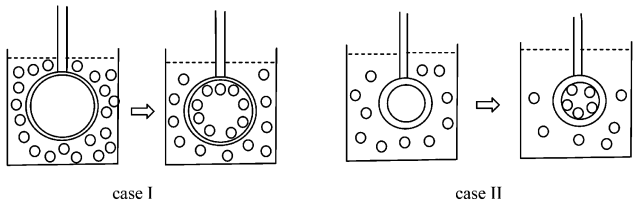

Fig. 2 Sensitivity increase by compartmentalization of receptor sites. A smaller compartment (case II) can in more dilute solution reach the same relative signal or size change as a larger compartment.

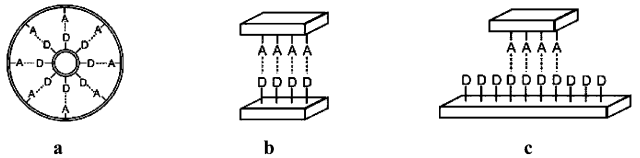

Fig. 3 Complexes with matching number of binding sites (chelate effect, cases **a, b**) and with a surplus of binding sites in the receptor (case **c**).

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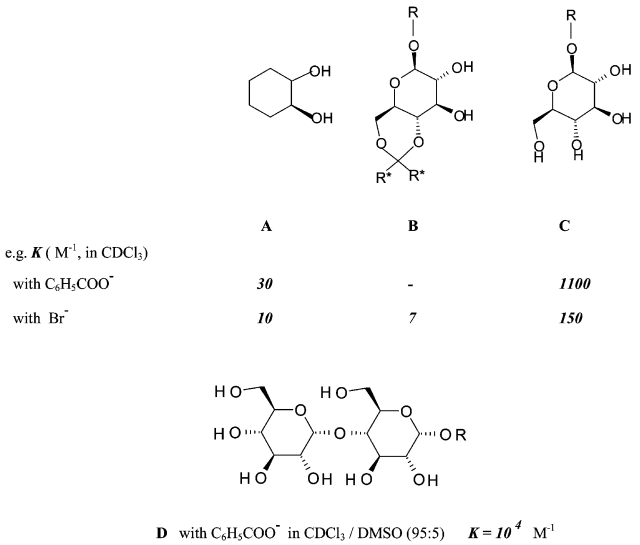

Fig. 4 Carbohydrate models **A**, **B**, **C** (R = *n*-decyl; R = CH_3) and a disaccharide **D** showing increased complexation constants *K* with an increasing number of OH-binding sites.

in the sense of the chelate effect (case c). Complexation measurements between anions and carbohydrate models bearing vicinal diols, which have an increased hydrogen bond strength and are ideally suited for 1 : 1 interactions with carboxylate groups[6] in chloroform as medium show a striking affinity increase with the number of binding groups (Fig. 4). It should be stressed that the bidentate carboxylate anion cannot simultaneously interact with more than two vicinal hydroxyl groups, and that under the measuring conditions 1 : 1 complexations were observed as evident from the perfect fit of the isotherms and from Job plots.[6] With the disaccharide **D**, only an approximate, yet again dramatically increased association constant *K* could be measured in presence of added DMSO.

In a related report it was observed that the presence of six chloro atoms in *e.g.* lindane makes such compounds as hydrogen bond bases as strong as *e.g.* an amine for complexation of *e.g.* a phenol.[7] The interpretation of these data was debated recently,[8] but it is obvious that a surplus of binding residues in a receptor can make it much stronger, an advantage which seems to be higher than that expected on statistical grounds alone. Similar affinity increases were also found with complexes between benzene derivatives with either biphenyl-shaped host compounds or with larger porphyrin derivatives as host; *e.g.* the complex of terephthalate and methylviologen[9] with $K = 2.5 \times 10^2$ M^{-1} was about 100 times weaker than that of terephthalate and tetrapyridiniumporphyrin ($K = 10^4$ M^{-1});[10] after correction for the ion pair contribution the stacking energy produced by the benzene moiety of terephthalate is either 4 or 9 kJ mol^{-1}.

The results show how synthetic receptors with high sensitivity can be designed by introducing a high density of recognition sites into a narrow space. This can be done in particular by implementation of a multitude of binding functions not only on surfaces, but also inside polymeric networks. Such arrangements can also contribute to the high sensitivity of cell surfaces for chemical signals, which in glycobiology has led to the postulation of a special cluster effect.[11] Life processes have long been assumed to have originated within coacervates[12] or microspheres.[13] The affinity enhancements described here could have secured within such compartments the accumulation of substrates available only in high dilution in the surroundings. Such concentration processes could then occur also in the absence of chelate effects, which would require the presence of already highly organized receptor units.

Notes and references

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