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Commentary

The different faces of poly(ethylene glycol)

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The paper by Sheth and Leckband (1) touches on an issue that has intrigued and bedeviled scientists in different fields for many years, namely, the very unusual interactions of poly(ethylene oxide) (PEO), $-[-CH_2-CH_2-O-]_n-$, or poly(ethylene glycol) (PEG), $-[CH_2-CH_2-O-]_n-CH_2-CH_2-OH$, and water. The unusual properties of PEO are found, and often made good use of, in polymer and surfactant technology, as well as in the ability of PEG to alter the interactions of cells and proteins with water and with each other.

The first sign that PEO is "unusual" can be sensed from it's unexpected water solubility or "hydrophilicity." Thus, poly(butylene oxide), $-[CH_2-CH_2-CH_2-O-]_n-$, having one more methylene (CH_2) group, is hydrophobic and insoluble in water, as might be expected. But so is poly(methylene oxide), $-[CH_2-O-]_n-$, which has one less methylene group.

Indeed, PEO appears to possess at least some hydrophobic character. For example, it forms thin monolayers at the air-water interface—a property that is commonly associated with amphiphilic molecules (such as surfactants and lipids) that have distinct hydrophilic and hydrophobic regions. In addition, molecules that have PEO or PEG groups attached to them such as surfactants, or "polymeric amphiphiles" possessing PEG headgroups, commonly exhibit a "lower consulate temperature" in their phase diagram. This means that when dissolved in water, small micellar clusters of these molecules aggregate or grow and then separate into two phases when the temperature T is raised, rather than lowered, which is the "normal" tendency. This effect has been attributed to the increasing attraction between the aggregates at higher temperatures, which is expected for the hydrophobic interaction because of its essentially entropic nature (for a purely entropic interaction we expect it to be proportional to T and, therefore, to increase with temperature). Indeed, in an earlier direct measurement of the forces between short-chained PEO groups using the SFA technique, Claesson *et al.* (2) found an attractive (adhesion) force between them that increased with temperature; but their results suggested that this was due more to the reduction in the magnitude and range of the repulsive forces between the PEO groups rather than to an enhancement of the attractive forces (which followed the expectations of the van der Waals forces). This could arise from the decreased excluded volume repulsion between these groups at higher temperature due to the reduced number of bound water molecules per ethylene oxide (EO) group (which are believed to bind via H-bonds to the –O– group of PEO). Thus, the increased attraction between certain EO groups at elevated temperatures may be due to a hydrophobic force or to a van der Waals force, but intimately coupled to the binding of water molecules to these groups.

All this, however, does not explain why PEO is so different from polymethylene oxide. Neither does it explain other unusual observations, such as why the properties of PEO are often found to depend critically on its molecular weight and, to a lesser extent, on the concentration. The interactions of higher molecular weight PEO are sometimes purely repulsive and well described by theories of inert polymer-mediated forces. In such cases two surfaces that expose PEO groups that are chemically bound to them at one end (end-grafted) will repel each other (3–6). But if the PEO is free in solution and repelled from the surfaces, it causes a "depletion" attraction between them, as occurs between cells, liposomes, or vesicles composed of lecithin bilayers, again in reasonably good agreement with theory (7–11).

But PEO, even when free in solution, can also be attracted to the surfaces of certain kinds of vesicles, cells or macromolecules, resulting in polymer adsorption that then gives rise either to a repulsion or to an attraction, via bridging, of the surfaces or vesicles—again depending critically on the temperature, molecular weight and concentration of the PEO. For example, while low molecular weight PEO generally induces cells or vesicles to adhere (depletion attraction), high molecular weight PEO causes them to repel (7–11). In the first case, the cells aggregate, in the second, they remain dispersed in solution ("stable"). The critical change-over molecular weight is between 10,000 and 20,000 Da, but nobody has managed to explain the underlying mechanism.

The reasons for the complex dichotomy between attraction and repulsion in the interactions of PEO, either as free polymer or when exposed at the surfaces of membranes or cells, are still not understood, especially when proteins are involved, and it is this issue that is the focus of the important paper by Sheth and Leckband (1). As they point out in the Introduction, there is a general feeling that PEO is essentially an inert hydrophilic polymer—repelling and being repelled by proteins—thereby providing a protective coat for any surface to which it is attached. This principle is behind the development of new biomimetic structures, such as the recently Food and Drug Administration-approved STEALTH liposomes that have great promise as drug delivery systems, and new classes of biocompatible materials.

What Sheth and Leckband have shown is that PEO may be repulsive only at long range, that is, only on initial contact; but when forced closer together against a layer of the protein streptavidin the interaction becomes attractive. Moreover, the shorter-ranged attraction is not weak but, although probably nonspecific, sufficiently strong to cause the adhesion or aggregation of proteins or of membranes exposing protein. These are potentially very important findings, both for our fundamental understanding of polymer–protein interactions and for the development of stable biomimetic systems. If the long-range interaction is repulsive but the short-range interaction is attractive, a system will remain stable only for a certain time. Depending on the repulsive energy barrier between the repulsive and attractive regimes, adhesion will always occur with time, even in the absence of an externally applied force pushing the two surfaces together. In other words, the dispersed, nonadhering, system is only kinetically stable, but not thermodynamically stable.

Using a SFA to measure the forces between two supported membranes at 1-Å distance resolution, Sheth and Leckband found that when phospholipid bilayer surfaces covered with streptavidin (a much studied receptor for biotin) interacted with another bilayer surface covered with PEO of M_r 2,000

(corresponding to 45 EO groups) the force between them was repulsive at large distances (>50 Å) and as expected from theories of electrostatic repulsive forces between these negatively charged surfaces. Closer in, the forces became even more repulsive, consistent with the expected "steric" repulsion of surface-bound polymer layers, although detailed comparison with theory was not made in this regime. Then, at even higher compressive pressures, corresponding to surface separations on the order of 25–50 Å (depending on the polymer coverage), the force changed to being attractive, and the surfaces adhered on being separated, but only after they were first pressed together under a large force. Sheth and Leckband discuss this and other possible interpretations of their results, but do not come down in favor of any one polymer model, feeling that more experiments are first required under different conditions and with different proteins. It appears, however, that more complex theories of polymer-mediated interactions would be needed to describe the interactions of a complex polymer such as PEO with proteins.

It is tempting, but probably dangerous, to generalize these results to all PEO protein systems, or to PEO with a different molecular weight (only PEO of M_r 2,000 was used in these studies), or even to a different pH. Thus, the pI of streptavidin is about 6.5, so that at the pH of the measurements (pH 7.0) one would expect there to be significant numbers of positively charged groups on the surfaces. Sheth and Leckband rule out the possibility that a simple Poisson–Boltzmann type of electrostatic interaction could explain the results. Such "charge regulated" interactions can give rise to an attractive electrostatic force at small separations even when the long-range force is repulsive. However, it is possible that discrete complementary charges on the two surfaces could bind to give rise to an attractive electrostatic force at small separations. If so, then this kind of electrostatic attraction would be expected to be very sensitive to pH (and calcium), especially near the pI or pK values of the charged residues on the protein surface.

On the other hand, hydrophobic patches on the protein surface may be responsible for the short-range attraction: these could induce intramolecular rearrangements to occur within the approaching PEO layer, resulting in the exposure of some hydrophobic groups to the hydrophobic patches on the protein. If this is the operating mechanism, then the exposed hydrophobic residues on the protein, rather than the charged or polar ones, would be implicated—a matter that should be easy to test.

Both types of interactions may not even be operating at true equilibrium (Sheth and Leckband found that a surprisingly long time was needed for the system to relax back to its original "state" after adhesion). If the attractive interaction is, indeed, a nonequilibrium one—the equilibrium interaction being purely repulsive—the strength of the adhesive forces would then be expected to depend on the rate of approach and separation (detachment) of the surfaces, and perhaps also on the contact time. This, too, could have very interesting implications, both for biological and nonbiological systems. The whole question of the mechanism of nonspecific polymer–protein interactions remains a confusing puzzle to which this work has added another valuable piece.

1. Sheth, S. R. & Leckband, D. (1997) *Proc. Natl. Acad. Sci. USA* **94,** 8399–8404.
2. Claesson, P., Kjellander, R., Stenius, P. & Christenson, H. K. (1986) *J. Chem. Soc. Faraday Trans.* **82,** 2735–2746.
3. Klein, J. & Luckham, P. (1984) *Macromolecules* **19,** 2007–2010.
4. Luckham, P. F. & Klein, J. (1985) *Macromolecules* **18,** 721–728.
5. Kuhl, T. L., Leckband, D. E., Lasic, D. & Israelachvili, J. N. (1995) in *CRC Handbook on Stealth Liposomes*, eds. Lasic, D. & Martin, F. (CRC, Boca Raton, FL), pp. 73–91.
6. Kuhl, T. L., Leckband, D. E., Lasic, D. D. & Israelachvili, J. N. (1994) *Biophys. J.* **66,** 1479–1488.
7. Boni, L. T., Stewart, T. P., Alderfer, J. L. & Hui, S. W. (1981) *J. Membr. Biol.* **62,** 65–70.
8. Boni, L. T., Stewart, T. P., Alderfer, J. L. & Hui, S. W. (1984) *J. Membr. Biol.* **80,** 91.
9. Boni, L. T. & Hui, S. (1987) in *Cell Fusion*, ed. Sowers, A. (Plenum, New York), p. 301.
10. Rupert, L. A. M., Engberts, J. B. F. N. & Hoekstra, D. (1988) *Biochemistry* **27,** 8232–8239.
11. Kuhl, T. L., Guo, Y., Alderfer, J. L., Berman, A., Leckband, D. E., Israelachvili, J. & Hui, S. W. (1996) *Langmuir* **12,** 3003–3014.