HYDROGEN BONDING IN CELLULAR COHESION

Malcolm N. JONES
Department of Biochemistry, University of Manchester, Manchester M13 9PL, England

Received 3 November 1975

1. Introduction

It is now well established that the surfaces of many cells are coated with a layer of complex carbohydrates arising from membrane bound glycoproteins and glycolipids. Apart from the specific biochemical functional properties they exhibit these surface molecules will influence the physico-chemical interactions between cells and will, in turn, have some influence on the cohesiveness of cells which is of fundamental importance in morphogenesis and metastasis [1,2]. At least two types of interaction between cell surfaces covered with carbohydrate can be envisaged. These are a long-range repulsive steric force arising from the excluded volumes of the polymeric chains and an attractive short-range force arising from hydrogen bonding between carbohydrate chain segments. There have been several theoretical studies of the steric repulsion problem [3–6] although its role in cellular cohesion has only recently been emphasised [7,8]. The possible involvement of hydrogen bonding in cellular cohesion has been pointed out in a general way [1,9,10] and also with specific reference to the problem of cell recognition [11] but there have been no attempts to estimate the magnitude of the interaction energies which might arise between cells due to hydrogen bonding. In this paper an equation for the attractive potential energy of interaction arising from short-range chemical interactions, such as hydrogen bonding is presented and the possible magnitude and importance of this type of attractive force in comparison with the classical electrodynamic forces is considered.

2. Theory

We consider firstly an isolated cell which is covered with a layer of glycoprotein and glycolipid material of thickness l. In this so-called 'glycocalyx' we have a number of monosaccharide residues each of which we can regard as a segment of a polymeric chain. We place the segments on a lattice of coordination number Z so that each segment occupies a lattice site of volume equal to that of a segment V_s. If there are n_s such segments associated with unit area of the cell surface then the site fraction of segments in the glycocalyx, x_s, will be given by the equation

$$x_s = \frac{n_s V_s}{l} \tag{1}$$

since the total number of sites associated with unit area of the cell surface, N, is simply l/V_s.

The number of segment-segment interactions in the glycocalyx, $n_{s\text{-}s}$, assuming random mixing of occupied and vacant lattice sites will be equal to the total number of nearest-neighbour sites, $x_s N(Z-2)+1$, multiplied by the fraction of those sites occupied by chain segments.

$$\therefore n_{s\text{-}s} = (x_s N(Z-2)+1)\, x_s \tag{2}$$

Note the addition of 1 to $x_s N(Z-2)$ corresponds to the sites at the free ends of the polymeric chains, the other ends being chemically attached to the membrane However, in general $x_s N(Z-2) >> 1$ so that neglecting the 1 and substituting for N and x_s in (2)

North-Holland Publishing Company – Amsterdam

$$n_{s\text{-}s} = \frac{n_s^2 V_s (Z-2)}{l} \quad (3)$$

If we now consider the domain between unit area of two interacting cells (each of which has n_s segments associated with them) such that the separation between the *membrane* surfaces is 2d, the site fraction of segments is now

$$x_s^i = \frac{n_s V_s}{d} \quad (4)$$

since the total number of lattice sites associated with unit area of the cell surfaces, N^i, is now $2d/V_s$. The total number of segment–segment interactions is now given by

$$n_{s\text{-}s}^i = (x_s^i)^2 N^i (Z-2) = \frac{2n_s^2 V_s (Z-2)}{d} \quad (5)$$

The only segment–segment interactions which are of importance in cohesion are those between different cells, hence to get this number, $n_{s\text{-}s}^d$, we must subtract from $n_{s\text{-}s}^i$ the number of self-interactions per unit area within the glycocalyx of the isolated two cells ($2n_{s\text{-}s}$).

$$\therefore n_{s\text{-}s}^d = n_{s\text{-}s}^i - 2n_{s\text{-}s} = 2n_s^2 V_s (Z-2) \left\{ \frac{1}{d} - \frac{1}{l} \right\} \quad (6)$$

If E_H represents the energy of formation of a hydrogen bond between two segments then the number of such bonds formed per unit area of cell surface will be given by equation (6) times the Boltzmann factor.

$$\therefore n_H = n_{s\text{-}s}^d \, e^{-E_H/kT} \quad (7)$$

and the potential energy of interaction per unit area will be given by

$$V_H = -E_H n_H$$

$$= 2n_s V_s (Z-2) E_H \left\{ \frac{1}{d} - \frac{1}{l} \right\} \exp.(-E_H/kT) \quad (8)$$

where the negative sign denotes that the energy is attractive. It follows from equation (8) that when $d=l$ so that the cell membranes are separated by exactly $2l$, $V_H=0$. That is when interpenetration between the glycocalyx of interacting cells cannot occur there is no attractive energy of interaction due to hydrogen bonding. It should be noted that equation (8) could be used to calculate a potential energy of interaction due to any type of short-range specific interaction between chain segments by introduction of the appropriate energy E.

3. Results and discussion

In order to calculate V_H as a function of membrane separation 2d, the parameters n_s, V_s l, Z and E_H are required. Of these n_s can be experimentally estimated, V_s estimated from molecular models (180×10^{-30} m^3 for a hexose segment) and Z can be put equal to 6 as is appropriate for a lattice model. The thickness of the glycocalyx, l, can be taken to be approximately equal to the root mean square end-to-end distance, $\langle \bar{r}^2 \rangle^{1/2}$, for polysaccharide chain of hexose units. This is given in metres by the equation [12]

$$\langle \bar{r}^2 \rangle^{1/2} = 515.9 \times 10^{-12} \, n^{1/2} \quad (9)$$

where n is the number of hexose units (segments) per chain. Calculation of n requires the molecular weight of the surface glycoprotein. For a random coil molecule $\langle \bar{r}^2 \rangle^{1/2}$ is approximately equal to the coil diameter [13]. Equation (9) gives the unperturbed end-to-end distance for a linear chain. Interaction with solvent will increase $\langle \bar{r}^2 \rangle^{1/2}$ on the other hand branching in the glycoproteins will decrease $\langle \bar{r}^2 \rangle^{1/2}$.

The formation of a single hydrogen bond between two segments requires the dehydration of the two participating OH groups. The process can be represented as

$$2 \text{ hexose } OH(H_2O)_m \rightarrow$$

$$\text{hexose } OH\text{---}\overset{H}{O} \text{ hexose } (H_2O)_{m-2} + 2H_2O$$

In this process two hydrogen bonds between the hexose OH groups and water are broken and one hydrogen bond is formed. If the two released water molecules remain free then an energy equal to approximately one hydrogen bond will be required. Thus E_H can be put equal to ~ 25 kJ mol^{-1} [14].

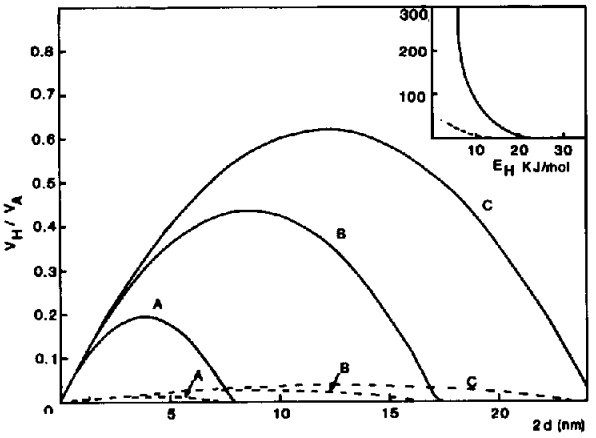

Fig.1. Ratio of attractive potential energy due to hydrogen bonding, to the electrodynamic potential energy of interaction (V_H/V_A) as a function of membrane separation (2d). Dashed curves for P388 cells, solid curves for CH 23 cells. A, B and C denote glycoprotein molecular weights of 10 000, 50 000 and 100 000 respectively. The inset shows the dependence of V_H/V_A on the energy of hydrogen bond formation, E_H for a glycoprotein molecular weight of 100 000.

In the plots shown in fig.1 the ratio of V_H to the attractive potential arising from the classical electrodynamic forces, V_A, has been plotted as a function of the membrane separation. The values of V_A were calculated from the equation

$$V_A = \frac{-A}{48\pi d^2} \qquad (10)$$

where A is the London–Hamaker constant. Equation 10 has frequently been applied to cellular interaction and values of A ranging from 0.6×10^{-20} to 1×10^{-22} J have been used in theoretical calculations [1]. Experimental values range from 0.2×10^{-20} J to 3×10^{-25} J [15]. A value of 0.8×10^{-20} J is taken here. Calculations were computed for two cell lines for which were measured the number of membrane bound glucose equivalent residues per cell by the anthrone method [16]. The two cell lines were a mouse lymphoma cell (P 388) and a Chinese hamster fibroblast (CH 23), both cell lines were cultured in vitro. Cell volumes were measured using a Coulter Counter and cell radii were calculated assuming the dispersed cells were spherical. From the surface carbohydrate levels and the cell surface areas values of n_s were obtained. These were 8.84×10^{-7} moles of equivalent glucose units m^{-2} for the P388 cells (radius 5×10^{-6} m) and 34.5×10^{-7} moles of equivalent glucose units m^{-2} for the CH 23 cells (radius 8×10^{-6} m). Three molecular weights of surface glycoproteins were assumed 10 000, 50 000 and 100 000.

The curves of V_H/V_A vs. 2d pass through maxima at $2d = l$. At very short distances $V_A >> V_H$ and at $d = l, V_H = 0$. It is important to note that experimentally measured values of the London–Hamaker constant and hence of V_A unavoidably include a contribution from hydrogen bonding if it occurs. For the P388 cells V_H makes a relatively small contribution to the overall attractive energy, being 4% at the most. For the CH 23 cells however V_H is a significant fraction of V_A, but it should be pointed out that for these fibroblastic cells the value of n_s could be an over estimate since no allowance has been made for folds and ruffles of the membrane surface. Inset in fig.1 is the effect of E_H on the ratio V_H/V_A for a surface glycoprotein molecular weight of 100 000. If E_H was reduced to 25% of a typical hydrogen bond energy the ratio increases by two orders of magnitude. Such a reduction in E_H is possible if the water molecules released from hexose OH groups do not remain free but participate in hydrogen bonding with the mass of water in the system.

4. Conclusion

It is shown that hydrogen bonding between monosaccharide segments in the glycocalyx of cells could contribute significantly to the total attractive potential energy of interaction between cells. It is however, problematical to what extent such bonding occurs. To the author's knowledge there is no evidence that sugars associate by hydrogen bonding in aqueous solution e.g. the activity coefficients of sucrose are very precisely interpreted up to 3 M in terms of a pentahydrate [17]. On the other hand some polysaccharides form gels in concentrated solutions [18], the bonding in these gels is co-operative hydrogen bonding in double helical structures between two polysaccharide chains as in the case of carrageenans or divalent metal ion induced fibrilation in alginates. More detailed studies on membrane glycoproteins are required before the

role of hydrogen bonding in cellular cohesion can be unequivocally decided.

Acknowledgement

The author thanks Mr R. Greig for carrying out the experimental work.

References

[1] Curtis, A. S. G. (1967) The Cell Surface, Logos-Academic Press, London.
[2] Cook, G. M. W. and Stoddart, R. W. (1973) Surface Carbohydrates of the Eukaryotic Cell. Academic Press, London and New York.
[3] Hesselink, F. Th., Vrij, A. and Overbeek, J. Th. G. (1971) J. Phys. Chem. 75, 2094–2103.
[4] Evans, R. and Napper, D. H. (1973) Kolloid-Z.u.Z. Polymer 251, 329–336.
[5] Smitham, J. B., Evans, R. and Napper, D. H. (1975) J. Chem. Soc. (Faraday I) 71, 285–297.
[6] Dolan, A. K. and Edwards, S. F. (1974) Proc. R. Soc. London, A337, 509–516.
[7] Maroudas, N. G. (1975) J. Theor. Biol. 49, 417–424.
[8] Maroudas, N. G. (1975) Nature, London. 254, 695–696.
[9] Pethica, B. A. (1961) Experimental Cell Research Supplement 8, 123–140.
[10] Jones, B. (1975) New Scientist 66, 552–554.
[11] Mayhew, E. (1974) J. Theor. Biol. 47, 483–484.
[12] Flory, P. J. (1953) Principles of Polymer Chemistry, pp. 421, Cornell University Press, New York.
[13] Tompa, H. (1956) Polymer Solutions. pp. 241, Butterworths, London.
[14] Pauling, L. (1940) The Nature of the Chemical Bond. pp. 333, Oxford University Press, London.
[15] Curtis, A. S. G. (1969) J. Embryol. Exptl. Morphol. 22, 305–325.
[16] Herbert, D., Phipps, P. J. and Strange, R. E. (1971) in: Methods in Microbiology, (Norris, J. R. and Ribbons, D. W. eds) 5B, pp. 265 Academic Press.
[17] Robinson, R. A. and Stokes, R. H. (1968) Electrolyte Solutions pp. 244, Butterworths, London.
[18] Rees, D. A. (1972) Biochem. J. 126, 257–273.