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MECHANISM OF DNA (SOUTHERN) AND PROTEIN (WESTERN) BLOTTING ON CELLULOSE NITRATE AND OTHER MEMBRANES

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SUMMARY

The transfer of DNA fractions from hydrophilic gels to nitrocellulose membranes (Southern blotting) which was soon followed by the description of an analogous procedure for RNA (Northern blotting), and somewhat later for proteins (Western blotting), has rapidly become an important separation and characterization method in molecular biology, genetic engineering, and immunological detection. Surface tension measurements have shown that the interfacial attraction between DNA and cellulose esters ($-\Delta G_{132}$) in aqueous media can be considerable. The weaker binding energy of proteins to cellulose nitrate and to cellulose acetate may be compared to hydrophobic interaction chromatography, as on account of the somewhat lower $|-\Delta G_{132}|$ values, it often is necessary to "fix" them more tightly onto nitrocellulose by using high salt concentrations. The binding energy of RNA to both cellulose esters also is rather low.

In addition to the effect of high ionic strength, the effect of adding methanol, and the effects of denaturation, heating and drying on the energy of attachment of the biopolymers to cellulose esters, have been studied.

Cationized nylon membranes have been advocated recently, especially for electrophoretic transfer of nucleic acids (in which process high salt concentrations cannot easily be used). With positively charged nylon membranes, the attachment mainly occurs through the electrostatic attraction between the strongly negatively charged nucleic acids (or proteins) and the positively charged membrane. Also, more apolar membranes (of polyvinyl difluoride) have been proposed, which manifest a strong interfacial (hydrophobic) attraction to all the above biopolymers (regardless of their electrostatic charge). However, with these two novel membrane types it is no longer possible to exploit the large difference in binding energy between DNA and RNA, which makes cellulose nitrate membranes so uniquely suited for RNA–DNA hybridization assays.

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INTRODUCTION

In 1965 it was first reported that cellulose nitrate filter membranes strongly adsorb single stranded DNA[1]. This observation led Gillespie and Spiegelman[2] soon thereafter to utilize the fact that DNA strongly binds to this type of membrane filters (while RNA, by itself, binds hardly at all to them), to develop an RNA–DNA hybridization system, with the DNA immobilized on these membranes.

In 1975 Southern[3] used this method, to transfer (blot) previously electrophoretically separated DNA fractions onto cellulose nitrate membranes, and to detect the specific sequences among these DNA fractions by RNA hybridization. This DNA separation and characterization method came to be called "Southern blotting". More or less logically, RNA-attachment to diazobenzyloxy-methylpaper[4] and related methods now are commonly called "Northern blotting". Finally, the blotting of protein fractions onto cellulose nitrate membranes, from sodium dodecyl sulfate polyacrylamide gel electrophoresis fractions was described in 1979[5]; this method soon became known as "Western blotting".

These blotting techniques have rapidly become indispensable isolation, "fixing" and characterization tools in molecular biology[6,7]. Meanwhile, Gillespie's statement of 1968: "Little is known about the mechanism of adsorption of single stranded DNA to membrane filters"[8] remains valid to this point. However, our recent approach toward solving the mechanism of interfacial (or hydrophobic) interactions between hydrophilic polymers and solid surfaces[9–11], can also be applied to the elucidation of the binding of DNA and protein and of the relative non-binding of RNA, to cellulose nitrate. We shall compare the mechanism of binding of DNA and protein to cellulose nitrate membranes with the mechanism by which these biopolymers can be blotted onto other materials, such as cellulose acetate, cationized nylon membranes or uncharged hydrophobic membranes.

THEORY

The surface thermodynamic principles pertinent to the energy of interaction between two different materials, whilst immersed in an aqueous medium, have been given in detail earlier[10,11], but for completeness' sake the necessary equations will be briefly outlined. It should be stressed that the major and crucial innovation of our treatment is the subdivision of non-covalent interactions into long range Lifshitz–van der Waals (LW) and short range (SR) interactions, where the latter are largely based upon the formation of hydrogen bonds[12]. Thus the total interaction in aqueous media (hitherto not infrequently called hydrophobic interactions) may be quantitatively described by[9,10]:

$$\Delta G^{IF} = \Delta G^{LW} + \Delta G^{SR} \tag{1}$$

where the superscript IF connotates the interfacial nature of these interactions. It should be kept in mind that whilst LW interactions have a long range nature, their magnitude at short range is considerably stronger than at long range. The major reason for treating LW and SR interactions separately lies in the fact that they obey different rules.

Long range (LW) interactions

The free energy of adhesion between two substances 1 and 2, at contact, *in vacuo*, is expressed by:

$$\Delta G_{12}^{adh} = \gamma_{12} - \gamma_1 - \gamma_2 \tag{2}$$

where γ_{12} is the interfacial tension between substances 1 and 2, and γ_1 and γ_2 are the surface tensions of these compounds. The free energy of adhesion between the same two substances, immersed in liquid 3, also at contact, is:

$$\Delta G_{132}^{adh} = \gamma_{12} - \gamma_{13} - \gamma_{23} \tag{3}$$

Eqns. 2 and 3 are valid for long range *and* for short range interactions, *at contact*. Interfacial tension components due to of Lifshitz–van der Waals (LW) interactions (comprising dispersion, orientation *and* induction forces[9,11,12]) can be obtained from LW surface tensions by the combining rule:

$$\gamma_{12}^{LW} = (\sqrt{\gamma_1^{LW}} - \sqrt{\gamma_2^{LW}})^2 \tag{4}$$

The LW surface tension of a solid 1 can be measured by contact angle (θ) determinations with completely apolar liquids 3, by using a variant of the Good–Girifalco–Fowkes equations:

$$\gamma_1^{LW} = \frac{\gamma_3 (1 + \cos \theta)^2}{4} \tag{5}$$

The values of γ^{LW} of LW liquids are known for many liquids[13], or can easily be measured by various standard methods.

Short range interactions

Unlike the LW interactions, which are mathematically symmetrical, the short-range interactions (*e.g.*, hydrogen bonds) are manifested as reciprocal, *i.e.*, donor–acceptor (acid–base) responses.

We can express γ^{SR} in a rigorous manner[10,11], by first introducing the components of γ^{SR}:

$$\gamma_i^{SR} = 2\sqrt{\gamma_i^+ \gamma_i^-} \tag{6}$$

See also Drago's approach in solution thermodynamics[14]. Here, we let γ^+ stand for the electron acceptor (Lewis acid) parameter of compound *i*, and γ^- for its electron donor (Lewis base) parameter*; γ^+ and γ^- are not necessarily equal, see below. A

* Hydrogen bonds can be treated as Brönsted acid–base (hydrogen donor–hydrogen acceptor) interactions or in the more general manner, of Lewis base–acid (electron acceptor–electron donor) interactions. Because of the Brönsted theory is included in the Lewis theory (but not *vice versa*) we use the Lewis theory here.

compound that can act both as a Lewis acid and a Lewis base is called bipolar. If it can act only as a Lewis acid or only as a Lewis base, it is called monopolar. Since compounds 1 and 2 may both be bipolar, *i.e.*, both may have electron donor and electron acceptor capabilities, the free SR energy of adhesion between substances 1 and 2 may be described as:

$$\Delta G_{12}^{SR} = -2\,(\sqrt{\gamma_1^+\gamma_2^-} + \sqrt{\gamma_1^-\gamma_2^+}) \tag{7}$$

A relationship in the form of eqn. 2 is also valid for ΔG_{12}^{SR}:

$$\Delta G_{12}^{SR} = \gamma_{12}^{SR} - \gamma_1^{SR} - \gamma_2^{SR} \tag{8}$$

or, rearranged:

$$\gamma_{12}^{SR} = \Delta G_{12}^{SR} + \gamma_1^{SR} + \gamma_2^{SR} \tag{8A}$$

Combining eqns. 8A, 6 and 7 (refs. 10 and 11):

$$\gamma_{12}^{SR} = 2\,(\sqrt{\gamma_1^+\gamma_1^-} + \sqrt{\gamma_2^+\gamma_2^-} - \sqrt{\gamma_1^+\gamma_2^-} - \sqrt{\gamma_1^-\gamma_2^+}) \tag{9}$$

or

$$\gamma_{12}^{SR} = 2\,(\sqrt{\gamma_1^+} - \sqrt{\gamma_2^+})\,(\sqrt{\gamma_1^-} - \sqrt{\gamma_2^-}) \tag{9A}$$

From the Young–Dupré equation:

$$\Delta G_{SL} = -\gamma_L\,(1 + \cos\theta) \tag{10}$$

where the subscripts S and L stand for solid and liquid. Using eqns. 6 and 7, the Young equation in terms of (LW + SR) can then be established:

$$1 + \cos\theta = \frac{2}{\gamma_L^{TOT}}\,(\sqrt{\gamma_S^{LW}\gamma_L^{LW}} + \sqrt{\gamma_S^+\gamma_L^-} + \sqrt{\gamma_S^-\gamma_L^+}) \tag{11}$$

where

$$\gamma_i^{TOT} \equiv \gamma_i^{LW} + \gamma_i^{SR} \tag{12}$$

Thus, using three different liquids with known γ_L^{LW}, γ_L^+ and γ_L^- values, γ_S^{LW}, γ_S^+ and γ_S^- should all be accessible by means of contact angle determinations. Thus, the contact angles of liquids 1, 2 and 3 are measured, on a solid, S. The values of $\gamma_{L_i}^{LW}$, $\gamma_{L_i}^+$ and $\gamma_{L_i}^-$ ($i = 1, 2, 3$) are substituted into eqn. 11 along with $\cos\theta$. The three equations with three unknowns are solved, for the values, γ_S^{LW}, γ_S^+ and γ_S^-.

In practice however, in many cases, this may not be so easily done: for most polar liquids the γ^+ and/or γ^- values are not yet known with any precision. γ^+ and

γ^- can be most easily determined in those cases where one of the interacting compounds is monopolar, *i.e.*, lacks either a γ^+ or a γ^- component[10,15]. The total γ^{SR} can be ascertained by contact angle measurement, preferably by using at least one apolar liquid, for obtaining γ^{LW} (eqn. 5), and by polar liquids for determining γ^+ and γ^-.

When γ_i^{LW}, γ_i^+ and γ_i^- ($i = 1, 2, 3$) are known we may write,

$$\Delta G_{132}^{SR} = 2\,[\sqrt{\gamma_3^+}\,(\sqrt{\gamma_1^-} + \sqrt{\gamma_2^-} - \sqrt{\gamma_3^-}) + \sqrt{\gamma_3^-}\,(\sqrt{\gamma_1^+} + \sqrt{\gamma_2^+} - \sqrt{\gamma_3^+}) - \sqrt{\gamma_1^+\gamma_2^-} - \sqrt{\gamma_1^-\gamma_2^+}] \quad (13)$$

To obtain ΔG_{132}^{TOT}, one must add to ΔG_{132}^{SR} the value of ΔG_{132}^{LW}, *i.e.*:

$$\Delta G_{132}^{LW} = \gamma_{12}^{LW} - \gamma_{13}^{LW} - \gamma_{23}^{LW} \quad (3A)$$

or

$$\Delta G_{132}^{LW} = -2\,(\sqrt{\gamma_3^{LW}} - \sqrt{\gamma_1^{LW}})\,(\sqrt{\gamma_3^{LW}} - \sqrt{\gamma_2^{LW}}) \quad (3B)$$

See eqn. 4.

For ascertaining γ_S^{LW}, hexadecane, diiodomethane or α-bromonaphthalene*, can be used, while for γ_S^{SR}, various polar liquids are available, including water. For determining γ_S^+, dimethylsulfoxide, may be used. This substance has a fairly high γ_L^- value (approximately 30 mJ/m², see below) and no γ_L^+ (refs. 10 and 16).

Electrostatic interactions

DNA has a relatively high negative surface (ζ) potential, and proteins have low to medium ζ-potentials; but cellulose nitrate and cellulose acetate have sufficiently low ζ-potentials to make the electrostatic repulsion energies between DNA (or protein) and cellulose nitrate or cellulose acetate membranes negligible, compared with the interfacial attractive energies here under study. However, with cationized (positively charged) membranes[17], an electrostatic *attraction* is the major driving force in DNA, protein (and also RNA) blotting.

EXPERIMENTAL

Materials

Human serum albumin (HSA; dried human Cohn Fraction V), and DNA (calf thymus, lot No. 506166), molecular weight ≃ 16000000, were obtained from Calbiochem (La Jolla, CA, U.S.A.); RNA (yeast) was obtained from BDH (Poole, U.K.); Lot No. 6972540A-259. IgG (human polyclonal IgG; dried Cohn Fraction II)

* It should however be mentioned that diiodomethane (γ^{LW} = 50.8 mJ/m²), being a relatively weak Lewis acid, has in addition a γ^+ component of about 1 mJ/m²: α-bromonaphthalene (γ^{TOT} = 44.4 mJ/m²) has a γ^{SR} component of about 0.9 mJ/m²; it has weak Lewis base as well as weak Lewis acid properties see Table III.

was obtained from GIBCO (Grand Island, NY, U.S.A.). All biopolymers were exhaustively dialysed against distilled water prior to use.

Cellulose nitrate membranes were obtained from Schleicher and Schuell (Keene, NH, U.S.A.); lot No. 6019/4. These membranes were too porous for contact angle measurement. After washing in distilled water, and drying, they were dissolved in methyl ethyl ketone, the solution deposited on glass slides, and dried. Cellulose acetate membranes were made according to van Oss and Bronson[18], and used for contact angle measurements, in the hydrated state. The material was also dissolved in methyl ethyl ketone and dried (as for cellulose nitrate).

The liquids used for contact angle measurements were all of highest and/or analytical grade. Their surface thermodynamic properties are enumerated in Table III.

Contact angle determination

The advancing contact angle is measured on the various hydrated and dry preparations with the different appropriate liquids. The advancing contact angle in the sense of Good[1] and Neumann and Good[20] was employed, *i.e.*, the contact angle observed immediately after the drop has stopped advancing. The liquid drop is deposited onto the horizontal substrate by means of a Gilmont 2-ml micrometer syringe (VWR Scientific; Rochester, NY, U.S.A.) and observed with a Gaertner (Chicago, IL, U.S.A.) goniometer fitted onto a Gaertner traveling X–Y micrometer attached to an optical bench illuminated with a diffuse light source (American Optical, Rochester, NY, U.S.A.).

For hydrated biopolymers a modification of the method first described by van Oss and co-workers[21,22] was used. Briefly, contact angles on hydrated biopolymers are measured on thick layers of protein collected upon anisotropic cellulose acetate membranes with pores too small to permit passage of the macromolecules[18], by means of ultrafiltration (under *ca.* 30 p.s.i. compressed nitrogen), with membranes of 42 mm diameter, starting with 20–25 ml of a solution of the biopolymers in distilled water. Ultrafiltration is continued until no further solvent passes through the membrane. The membrane is then allowed to dry at room temperature, until a constant contact angle with drops of water is obtained. At that "plateau value" (advancing) contact angle measurements are then performed with water and other liquids. The time during which the plateau value persists can be much prolonged by supporting the membrane (which has the hydrated biopolyer on its upper surface) with its lower surface on a slab of 1% agarose in water, about 1 cm thick[22,23].

The same biopolymer solution can be used to make layers of dried material on glass (microscope) slides by depositing *ca.* 1 ml solution per slide, allowing the solution to spread over most of the slide's surface, and air-drying the slides for one or two days at ambient temperature, after which the slides are further dried by storing them in a vacuum desiccator (partly filled with, *e.g.*, calcium sulfate), for two or more days.

RESULTS

Table I shows the contact angles found with a number of liquids, on DNA, RNA, two representative proteins (HSA and IgG), (in the hydrated as well as the

TABLE I

CONTACT ANGLES FOUND WITH VARIOUS LIQUIDS

		Water	*Hexa-decane*	*Dimethyl sulfoxide*	*α-Bromo-naphthalene*	*Diiodo-methane*	*Glycerol*
DNA	Hydrated	12°	18°	17°*	25°	30.5°	9°
	Dry	57.4°		0	28.5°	42°	52°*
RNA	Hydrated	0	22.3°	6°	41°	46.5°	10°*
	Dry	16.5°*		42.7°	41°	44°	75°*
HSA	Hydrated	12.8°**	9.7°**				
	Dry	63.5°***		19.8°***	23.2°***	34°***	62°***
IgG	Hydrated	23°**	10°**				
	Dry	71.2°***		16°***	22°***	34°***	62°***
Cellulose nitrate	Hydrated	60°		30°	8°*	37°	45.5°
	Dry	67.5°*		30.8°	7.5°	27°	62.5°
Cellulose acetate	Hydrated	53.7°		45.3°	30.5°	33°	52°
	Dry	54.5°		30°	32°	55°	62°

* Decreases.
** From refs. 9 and 22.
*** From ref. 23.

dry state) and cellulose nitrate and cellulose acetate. The various surface tension components following from these contact angles are given in Table II. In Table III the surface tension data are listed for the liquids used in measuring these contact angles. Table II shows the surface tension components for the nucleic acids, the proteins and the cellulose esters, derived from Table I.

TABLE II

SURFACE TENSION PARAMETERS OF DNA, RNA, HSA, IgG, CELLULOSE NITRATE AND CELLULOSE ACETATE, DERIVED FROM THE DATA OF TABLE I (IN mJ/m²)

		γ^{LW}	γ^{SR}	γ^{+}	γ^{-}	γ^{TOT}
DNA	Hydrated	26.1*	45.5	36**	14.4***	71.6
	Dry	39§		40	very small	(39)
RNA	Hydrated	25.5*	48	32***	18**	73.5
	Dry	37.5§		0	50.4	37.5
HSA	Hydrated	27*	44.6	27.6***	18**	71.6
	Dry	41§		0	15	41
IgG	Hydrated	27*	40.85	23.2***	18**	67.85
	Dry	42.5§		0	9.2	42.5
Cellulose nitrate	Hydrated	38§§		0	18.3	38
	Dry	38§§		0	12.9	38
Cellulose acetate	Hydrated	38§§§		0	23.4	38
	Dry	38§§§		0	23.0	38

* From θ of hexadecane.
** See text.
*** From γ^{SR} and γ^{-}, see eqn. 6.
§ From θ of diiodomethane.
§§ From θ of dimethyl sulfoxide.
§§§ From θ of α-bromonaphthalene.

TABLE III

SURFACE TENSION PARAMETERS OF LIQUIDS USED IN CONTACT ANGLE MEASUREMENTS (IN mJ/m²)

	γ^{LW}	γ^{SR}	γ^{+}	γ^{-}	γ^{TOT}
Water	21.8*	51*	36**	18**	72.8*
Hexadecane	27.5	0	0	0	27.5***
Dimethylsulfoxide	44	0	0	30	44***
α-Bromonaphthalene	43.54	0.86§	0.43§	0.43§	44.4***
Diiodomethane	50.8	0	1	0	50.8*
Glycerol	34*	30*	8§§	28§§	64*

* Ref. 12.
** Ref. 10.
*** Ref. 13.
§ From the interfacial tension between water and α-bromonaphthalene of 42.1 (ref. 28); for lack of other data, the value for γ^{+} is equated with that of γ^{-}.
§§ Ref. 11.

Table IV shows the free energies of adhesion ΔG_{132} and of hydrated and dry DNA, RNA, HSA and IgG, with cellulose nitrate and cellulose acetate (using the surface tension components of Table II, and eqns. 1, 14, 4 and 13) as well as ΔG_{12}, using eqns. 2 and 9.

DNA (dry) behaves like a Lewis acid, *i.e.*, it mainly appears to manifest a γ^{+}, while RNA (dry) as well as HSA (dry) and IgG (dry) behave mainly like Lewis

TABLE IV

FREE ENERGIES OF ADHESION ΔG_{132} AND ΔG_{12} OF HYDRATED AND DRY NUCLEIC ACIDS AND PROTEINS TO CELLULOSE NITRATE AND CELLULOSE ACETATE (IN mJ/m²)

The binding energies of hydrated materials are set in bold face type to indicate that these are the decisive values for binding.

		ΔG_{132}	ΔG_{12}
DNA on	Hydrated	**−6.7**	−114.6
Cellulose nitrate	Dry	−58.1	−122.4
DNA on	Hydrated	**−6.9**	−76.0
Cellulose acetate	Dry	−40.9	−137.6
RNA on	Hydrated	**−1.1**	−48.4
Cellulose nitrate	Dry	+22.0	−75.5
RNA on	Hydrated	**−0.8**	−69.4
Cellulose acetate	Dry	+36.6	−75.4
HSA on	Hydrated	**−1.5**	−87.0
Cellulose nitrate	Dry	−17.6	−78.9
HSA on	Hydrated	**−0.7**	−70.3
Cellulose acetate	Dry	−3.0	−78.9
IgG on	Hydrated	**−1.5**	−64.7
Cellulose nitrate	Dry	−27.8	−80.4
IgG on	Hydrated	**−0.2**	−69.7
Cellulose acetate	Dry	−13.5	−94.0

bases, *i.e.*, they principally manifest their γ^- components. In the hydrated form this monopolar behavior is much attenuated through hydration. In the case of DNA (hydrated) we must take into account orientation of the hydrating water dipoles, to the effect that, with mainly a γ^+ at the core of each hydrated molecule, the hydrated entity still will have the major γ^+ of the water dipole predominating, *i.e.*, the $\gamma^+ \approx 36$ mJ/m^2 (refs. 10 and 16). The γ^- of the hydrated molecule then follows directly from the γ^{SR} of 45.5 mJ/m^2 (eqn. 6); *i.e.*, $\gamma^- = 14.4$ mJ/m^2. For RNA, HSA and IgG the opposite is true, *i.e.*, their hydrated γ^- is *ca.* 18 mJ/m^2, while their γ^+ follow from their γ^{SR} values (and eqn. 6). The preponderant monopolar γ^+ property of DNA and the equally monopolar γ^- property of RNA, and of most proteins[21], are properties of the integral polymers, and are not directly connected to the specific hydrogen-bonding links DNA (and RNA) monomer base-pairs mutually can engage in.

DISCUSSION

Southern (DNA) blotting

From the fact that the ΔG_{132} values for hydrated materials are around -6.8 mJ/m^2 for DNA, *vs.* around -1 mJ/m^2 for the other hydrated substances, it is clear (Table IV) that hydrated DNA should bind more strongly to cellulose ester membranes than hydrated RNA or proteins. Indeed, RNA binds even more weakly to cellulose nitrate than proteins. The reason for the peculiar affinity of DNA for these cellulose esters clearly lies in the fact that DNA is strongly endowed with a preponderant γ^+ component, while the cellulose esters (and the proteins and RNA) have stronger γ^- components. This increases the attraction between DNA and the cellulose esters, and tends to diminish the attraction between these membranes and RNA or proteins. At around -6 to -7 mJ/m^2, the attraction of hydrated DNA to cellulose esters would reach $-1.5\ kT$ (where k is the Boltzman constant and T the absolute temperature), if the contactable area a DNA molecule can present to the membrane surface is of the order of 100 Å^2 or more. Binding would be assured from that point on. This explains why DNA of higher molecular weight binds better than small strands[8], and why, in order to promote binding of small DNA strands, the addition of salt is needed[8], as the addition of salt results in partial dehydration (compare the values for ΔG_{132} for hydrated with those for dry DNA, Table IV), and thus in enhanced binding[10,22].

Western (protein) blotting

From the energies of binding (ΔG_{132}) of hydrated proteins (Table IV), it would appear that the binding of proteins of relatively low molecular weight onto cellulose nitrate would not be very permanent as, with *ca.* 200 Å^{2*} contactable surface area, a binding energy of *ca.* -1.5 mJ/m^2 would only amount to about -0.75 kT, or only about half the binding energy needed to attain permanency. However, the addition of salt (*e.g.*, to at least double or triple the amount normally present in physiological

* We have postulated earlier that the most likely contactable surface area of proteins, for protein-protein interactions, for smooth, hydrated proteins, must be of the order of *ca.* 100 Å (refs. 10 and 22); however, the contactable area of proteins *vis-a-vis* irregular pore surfaces is more likely around twice that value.

saline solutions), would greatly increase the binding energy through partial dehydration (in the direction of ΔG_{132} of the dry proteins; see Table IV), sufficiently to ensure a more lasting bond[10,17].

It is of interest to determine what the influence of 20% methanol would be on the binding of protein (*e.g.*, HSA, hydr.) to cellulose nitrate, as methanol is often used to enhance protein binding, while it also decreases the detrimental influence of sodium dodecyl sulfate on the binding[6,17]. One can relatively easily calculate how the admixture of 20% (v/v) methanol affects ΔG_{132}. As one cannot go by the measured γ^{TOT} of methanol–water (20:80) of 46.05 mJ/m^2 [24], which value is much too low because of the strong surface activity of all alcohols (including methanol) we have to calculate the actual γ^{TOT} ($= -\frac{1}{2}\Delta G^{coh}$; where ΔG^{coh} is the energy of cohesion of the liquid) of methanol–water (20:80). As the surface tension of pure methanol is 22.5 mJ/m^2 and of water 72.8 mJ/m^2 (ref. 16), a 20% (v/v) mixture should have a $\gamma^{TOT} = 62.7$[4] and a γ^{LW} of 21.95. This yields a γ^{SR} of 40.79 mJ/m^2. As, in analogy with ethanol[16], methanol should have a γ^- of at least 25 mJ/m^2, and no significant γ^+, the γ^- of 20% methanol would be *ca.* 19.4 mJ/m^2, and from the γ^{SR} of 40.79 (and eqn. 6), the γ^+ of the mixture would be *ca.* 21.4 mJ/m^2. In methanol–water (20:80) the interaction energy of HSA (hydrated) with cellulose nitrate then ΔG_{132} would be *ca.* -2.9 mJ/m^2 (eqns. 14 and 13), which is about twice the value found without methanol (Table IV). This computation agrees well with the known enhanced degree of protein binding through the admixture of methanol.

RNA-binding

The energy of RNA-attachment to the cellulose esters (Table IV) is the lowest of all biopolymers studied. This is due to the pronounced monopolar properties of RNA (as measured in the dry state), with a high γ^- value (Table II), which follows from the contact angle measurements, in particular from the unusually low contact angle with water on dry RNA (Table I). RNA (hydrated) thus is less strongly attracted (in water) to the monopolar (also strongly γ^-) cellulose nitrate than proteins, and much less strongly attracted to such monopolar (γ^-) cellulose esters than DNA, which is preponderantly monopolar with the opposite (γ^+) sign. For the original hybridization assays (Southern blotting) it therefore was extremely advantageous to use a membrane that strongly binds DNA, but that would not bind RNA to any significant extent, thus allowing only specific RNA to combine with the bound DNA[3]. From Table IV it would not appear that simply drying would at all enhance the attachment of RNA to cellulose nitrate or acetate (the positive ΔG_{132} values would support the opposite). It is therefore not surprising to note that to achieve RNA binding to cellulose nitrate, drastic denaturation of the RNA is required[7].

Influence of drying

Upon comparison of the ΔG_{132} values for the binding of hydrated *vs.* dry biopolymers to the cellulose esters, (in water) it would appear that with DNA, as well as with proteins, the binding energy increases upon drying (for RNA, see the preceeding section). When *total* dehydration is practiced, one may expect the binding energies to become reflected by the ΔG_{12} values, which are much more strongly negative (*i.e.*, attractive) than the ΔG_{132} values (which still presuppose the presence of interstitial water). However, when re-wetted the binding could weaken consider-

ably, reverting, in all reversible cases, to the ΔG_{132} values for the hyrated biopolymers. Thus for *permanent* binding, even upon reimmersion in aqueous media, the biopolymer should be irreversibly denatured (especially in the case of RNA[7,8]), or at least thoroughly heated ("baked"), *e.g.*, at 80°C for 2 h, in the cases of DNA[7], and various proteins.

Comparison between cellulose nitrate and cellulose acetate

In the cases of RNA and of the proteins studied, binding to cellulose acetate is definitely weaker than to cellulose nitrate. This is one of the reasons why, for already 30 years, cellulose acetate has proved an almost ideal support for electrophoretic analysis of serum proteins, as undesirable protein adsorption to the carrier remains at a minimum[25,26]. For DNA, according to Table IV, there do not seem to be significant differences in binding energies to cellulose nitrate or acetate. But for proteins the difference is crucial. Western blotting is possible with cellulose nitrate, and difficult or even almost impossible with cellulose acetate. For RNA the difference is also striking, and the even smaller aspecific binding of RNA to cellulose acetate, may well make this a more advantageous membrane to use for RNA–DNA hybridization.

Binding to other membranes

Recently more purely hydrophobic membranes have been proposed, made of polyvinylidene difluoride[27]. If we assume the γ^{LW} of this membrane to be *ca.* 19 mJ/m^2 (ref. 9), and its γ^+ and γ^- to be nonexistent, then its binding energies ΔG_{132} with hydrated biopolymers (eqns. 14 and 13) would be: DNA (hydr.) -5.1, RNA (hydr.) *ca.* -2.6, HSA (hydr.) *ca.* -2.9 and IgG (hydr.) *ca.* -9.7 mJ/m^2. Thus, except for DNA, where no improvement would occur, a strong increase in binding energy can be predicted: 2.5 times for RNA; 2 times for HSA, and 6.5 times for IgG. The principal drawback of these membranes would lie in the strong (aspecific) binding of RNA, which would make the interpretation of DNA–RNA hybridization studies much more difficult. The addition of methanol would *decrease* the binding to all biopolymers; the addition of salt would *enhance* their binding.

Another new approach is the use of cationized (nylon-based) membranes[17]. These membranes bind strongly with all negatively charged biopolymers (*i.e.*, stronger with DNA, RNA and HSA, and somewhat more weakly with IgG; all at roughly neutral pH). Here again we have the same drawback for DNA–RNA hybridization, as RNA binds as avidly to these membranes as DNA. Methanol would *enhance* the binding (by decreasing the dielectric constant), but salt would *decrease* it.

CONCLUSIONS

Due to the fact that DNA is biased towards electron acceptor behavior and the two cellulose esters studied towards the electron donor component of the surface tension, a sizeable bond is formed between the two types of material. As RNA and all proteins studied to date, tilt toward the electron donor surface tension component, as do the cellulose esters, RNA and the proteins in question manifest a much weaker attraction to cellulose ester membranes than DNA.

It can be predicted that drying would enhance the bond strength in all cases, except for RNA, where not even the most drastic desiccation would result in attachment of undenatured material. Actual denaturation leads to increased hydrophobicity in all cases and thus should lead to stronger binding of all materials. Addition of neutral salts leads to dehydration, and thus to increased hydrophobicity, at least of DNA and the proteins and therefore also leads to their enhanced binding. Admixture of (*ca.* 20%) methanol, which changes the electron-donor/electron-acceptor proportion of the solvent, likewise increases the binding energy.

While DNA binds about equally strongly to cellulose nitrate and cellulose acetate, RNA and the proteins attach more strongly to cellulose nitrate than to cellulose acetate, mainly because the latter's electron donor surface tension component is somewhat larger than that of cellulose nitrate.

The fact that DNA only binds to these membranes when it has reached a minimum degree of polymerization is easily understood when the binding energies are expressed in terms of kT. Only when -1.5 kT has been reached, can the thermal, or Brownian, dissipation energy be overcome. For -1.5 kT to be attained, a minimum contactable surface area of *ca.* 100 Å^2 must be available. Small oligomers of DNA do not present that amount of minimum contactable surface area.

Membranes made of materials other than cellulose esters bind DNA, RNA and proteins in an entirely different way, but they cannot discriminate between DNA and RNA in the manner of the cellulose esters, which permits the latter to be uniquely suited for DNA–RNA hybridization. It would follow from our data that cellulose acetate might be even somewhat more effective for hybridization assays than cellulose nitrate, on account of its still smaller aspecific attraction for RNA, combined with the same binding capacity to DNA.

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