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Polyacrylamides Bearing Pendant α-Sialoside Groups Strongly Inhibit Agglutination of Erythrocytes by Influenza Virus[1]

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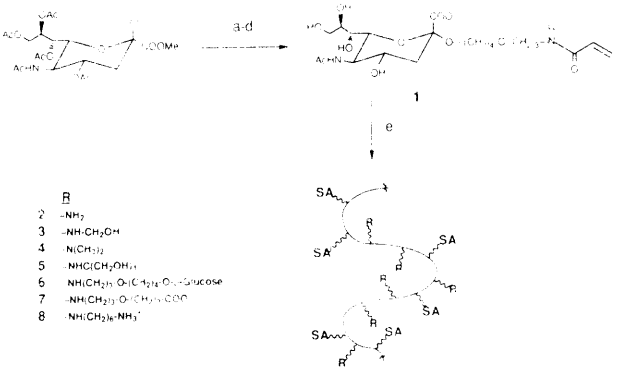
The initial step of invasion of a mammalian cell by influenza virus is the binding of the viral membrane protein hemagglutinin (HA) to sialic acid (SA) residues of cell surface glycoproteins and glycolipids.[2] Tight-binding inhibitors of this association are potential inhibitors of influenza infection. Systematic examinations of monomeric derivatives of sialic acids have not, to date, revealed compounds binding significantly more tightly to HA than α-glycosides of sialic acid.[3,4]

Although the binding of HA to α-sialosides is weak ($K_D \sim 2 \times 10^{-3}$ M),[3] the binding of influenza virus to cells appears to be strong. The qualitative difference between the strength of interaction between monomeric HA and methyl α-sialoside, and that between virus and cell surface, probably reflects the polyvalency of the latter interaction.[5] This inference is supported by the observation that certain glycoproteins, especially α_2-macroglobulins, having high contents of sialic acid are strong inhibitors of virus-induced agglutination of erythrocytes.[6,7] Of the known sialic acid containing glycoproteins, only a few are capable of protecting erythrocytes from viral agglutination, and it is difficult to pinpoint the origin of this activity. We believe that the number and accessibility of sialic acid groups in these glycoproteins play key roles.

The structures of these complex, naturally occurring, polyvalent hemagglutination inhibitors are largely unknown, and it is impractical to prepare close analogues of them by synthesis, or to study relations between their structure and strength of inhibition. We therefore sought practical routes to synthetic macromolecules to which sialic acid groups could be attached, and in which composition and strucxture could be varied readily. Here we report that such substances can be prepared conveniently by free-radical copolymerization of **1**, an acrylamide derivative of sialic acid, with acrylamide and its derivatives (Scheme I). The most active of these copolymers are powerful inhibitors of hemagglutination by influenza virus.[8]

Polymerizations followed standard procedures.[9] We have not characterized these polymers fully, but dialysis of representative

Scheme I. Synthesis of Copolymers of **1** and Acrylamides[a]



[a] (a) $HO(CH_2)_4O(CH_2)_3NHCbz$, Ag-salicylate, C_6H_6, 25 °C, 3 days; (b) 1 N NaOH, 25 °C, 12 h; (c) H_2/5% Pd–C, MeOH, 25 °C, 6 h; (d) *N*-(acryloyloxy)succinimide, Et_3N, H_2O, 25 °C, 12 h; (e) CH_2=CHCOR, 4,4′-azobis(4-cyanopentanoic acid), *hν* (365 nm), 25 °C, 5 h.

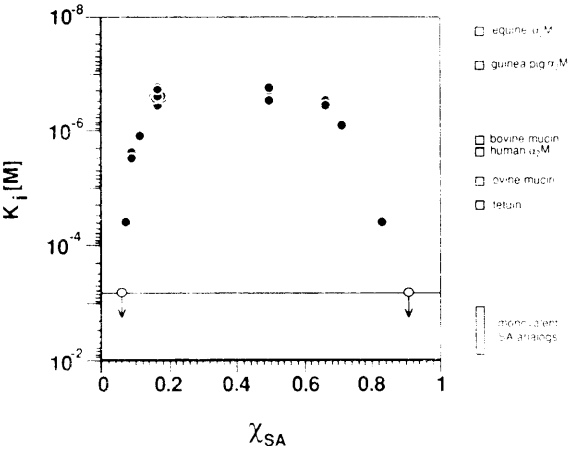


Figure 1. Inhibition of hemagglutination of erythrocytes by poly(**1**-*co*-acrylamide). The inhibition constant, K_i, is calculated on the basis of sialic acid groups in solution. χ_{SA} is the mole fraction of **1** in the mixture of **1** and acrylamide used in forming the polymer. The cluster of data at $\chi_{SA} = 0.17$ corresponds to 10 independent experiments producing the values of $K_i = 1.8 \times 10^{-7}$ (2×), 3.0×10^{-7} (7×), and 3.6×10^{-7} (1×). The reference data listed at the right margin of the figure for proteins and analogues of SA are taken from refs 4 and 6. We have confirmed the values of bovine mucin and fetuin independently. Polymers having values for $K_i > 6.25 \times 10^{-4}$ M (the horizontal line in the figure) were not examined quantitatively, and the points (○) at $\chi_{SA} = 0.063$ and 0.91 represent lower limits.

Table I. Values of K_i for Copolymers Prepared from 1:1 Molar Mixtures of **1** and Acrylamides

acrylamide	K_i, M
2	3.0×10^{-7}
3	3.0×10^{-7} [a]
4	2.5×10^{-6}
5–**8**	$>6 \times 10^{-4}$

[a] This copolymer is only partially soluble.

(1) This research is part of a collaboration with J. Skehel, J. R. Knowles, M. Karplus, and D. C. Wiley and was supported by the NIH, Grant GM 39589. A.S. is a Postdoctoral Fellow of the Swiss Academy of Sciences.
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(9) Compound **1** and acrylamide (or an analogue) were mixed in aqueous solution at pH 7.0 (with a total concentration of acrylamide moieties of 1.0 M) containing initiator (0.02 M). The solution was deoxygenated by passing argon through it, and polymerization was initiated by using a UV lamp (365 nm).

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samples using a membrane with a MW cutoff of 10000 completely retained the material that inhibited hemagglutination. The activity also showed no fractionation after chromatography on a Sephadex G-10 column. We conclude that the molecular weights are high and that the inhibitory activity is not influenced by small variations in molecular weight. Treatment of poly(**1**-*co*-acrylamide) with neuraminidase (EC 3.2.1.18) abolished its activity. This control indicates that the α-sialoside groups in this polymer are substrates for this enzyme and demonstrates that the activity of the polymer in inhibiting hemagglutination is due to these groups.

Figure 1 summarizes the inhibition by these polymers of the hemagglutination of chicken erythrocytes by influenza X-31 virus.[8] *The values of K_i are calculated on the basis of the concentrations of sialic acid moieties in the solutions*, not of the polymer chains to which they are linked.[10] These values are thus directly comparable with one another and with values of monomeric and oligomeric derivatives of sialic acid. The most important of these data indicate that sialic acid groups incorporated into the polymer formed from **1** and acrylamide in ratios [**1**]/[acrylamide] $\simeq$ 0.2–2 are more effective than methyl α-sialoside in inhibiting hemagglutination by $\sim 10^4$–10^5 and are only approximately a factor of 10 less effective than the sialic acid groups in equine α_2-macroglobulin. *Polyvalent derivatives of sialic acids are thus much more effective than monomeric ones in inhibiting hemagglutination.*

We have prepared a substantial number of copolymers of **1** with N-substituted acrylamides and tested them for their ability to inhibit hemagglutination; a few examples are summarized in Table I. On the basis of these data, we infer that bulky groups and charged groups R (Scheme I) interfere with binding.

We rationalize the bell-shaped curve in Figure 1 using an argument based on a competition between entropy and efficiency of utilization of sialic acid groups. At low values of χ_{SA}, the distance between sialic acid groups is large and the entropic advantage of binding resulting from connecting them is small; at high values of χ_{SA}, only a few sialic acid groups can bind to the HA sites, and the rest, those between the bound groups, do not participate in binding. The value of χ_{SA} may also influence the conformation of the polymer and nonspecific interactions with the virus in ways important to its activity in binding.

This work demonstrates a new strategy for the design and synthesis of compounds that inhibit binding of influenza virus to the surface of cells.[11] This strategy is based on polyvalent polymeric inhibitors able to compete with the polyvalent virus–cell interaction.[12] It is possible, by basing the syntheses of these inhibitors on copolymerization reactions of relatively readily synthesized monomers, to prepare macromolecule structures easily, albeit with incomplete knowledge of the distribution of groups along the backbone of the polymer and in space. This strategy for the preparation of polyvalent binding agents should be generally applicable to the generation of a wide range of tight-binding agents directed toward receptors or ligands of which multiple copies are present on the surface of the target cell, microorganism, or virus.

Note Added in Proof. Results similar to those reported here have been obtained in independent work by Matrosovich et al.[13]

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(10) The hemagglutination inhibition constant K_i is defined as the lowest concentration of sialic acid residues that completely inhibits agglutination of erythrocytes by influenza virus at 4 °C under the assay conditions defined in ref 8.

(11) A related approach has been used to generate polyvalent carbohydrate antigens: Kochetkov, N. *Pure Appl. Chem.* **1984**, *56*, 923–938. Roy, R.; Laferiere, C. A. *Carbohydr. Res.* **1988**, *177*, c1–c4.

(12) It remains to be established whether inhibition of hemagglutination correlates with inhibition of infection in vivo. Ellens et al. have suggested that the minimum unit required for fusion is small (several HA trimers): Ellens, H.; Bentz, J.; Mason, D.; Zhang, F.; White, J. M. *Biochemistry* **1990**, *29*, 9697–9707.

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