The partial characterization of the binding of avidin–biotin complex to rat liver plasma membrane

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Rat liver plasma membrane and rat intestinal mucosa plasma membrane were examined for their ability to bind avidin–[³H]biotin complex. Avidin–[³H]biotin complex bound specifically to liver plasma membrane with a K_d of approx. 35 nM and B_{max} of 136 pmol/mg of membrane protein. The receptor on the liver plasma membrane was exposed by Pronase, sensitive to tryptic hydrolysis and insensitive to neuraminidase. The optimum pH of specific binding was less than 6.0. Simple carbohydrates, mannan and ovalbumin did not inhibit specific binding. Periodate-treated or α -mannosidase-treated avidin was able to inhibit binding of either avidin–[³H]biotin complex or untreated avidin to plasma membrane. [³H]Avidin containing methylated lysine residues did not bind to rat liver plasma membrane. There was no specific binding of the avidin–biotin complex to rat intestinal mucosa plasma membrane.

Avidin is a tetrameric glycoprotein that binds biotin with a K_d of about 1 fM (Green, 1975). Each monomer binds biotin independently with no cooperativity between subunits. The oligosaccharide chain of avidin contains mannose and *N*-acetylglucosamine and its structure is of the ovalbumin type. We have previously demonstrated that the avidin–[³H]biotin complex was internalized by HeLa cells by receptor-mediated endocytosis (Dakshinamurti & Chalifour, 1981). As avidin is not a naturally circulating protein the possibility that it mimics a natural ligand and that the oligosaccharide chain on avidin might be involved in this binding was considered. Rat liver plasma membrane has been found to contain receptor systems specific for glycoproteins based on the terminal carbohydrate on their oligosaccharide chains (Neufeld & Ashwell, 1980). In the present study we have examined the binding of avidin–biotin complex to rat liver and intestinal mucosa plasma membrane.

Experimental

Materials

Earle's balanced salt solution was purchased from Flow Laboratories Inc., Mississauga, Ont., Canada. 'Trypsin-TPCK' (1-chloro-4-phenyl-3-L-toluene-*p*-sulphonamidobutan-2-one-treated trypsin) was obtained from Worthington Biochemical Corp., Freehold, NJ, U.S.A.; Pronase P, from *Streptomyces griseus*; avidin, from egg white; d-biotin, propionyl-

CoA, acetyl-CoA, ATP (disodium salt), α -D-mannosidase (EC 3.2.1.24) from jack beans and sucrose (density-gradient grade) were purchased from Sigma Chemical Co., St. Louis, MO, U.S.A. $\text{NaH}^{14}\text{CO}_3$ (sp. radioactivity 40–60 Ci/ μ mol) and NaB^3H_4 (sp. radioactivity 64 Ci/nmol) were purchased from New England Nuclear Corp., Boston, MA, U.S.A. (+)-[³H]Biotin (sp. radioactivity 2.4 Ci/nmol) was a gift from Hoffman-LaRoche Inc., Nutley, NJ, U.S.A. NCS tissue solubilizer and OCS scintillation cocktail were purchased from Amersham-Searle Corp., Oakville, Ont., Canada.

Uptake of avidin–[³H]biotin in vivo by rat liver cells

Avidin–biotin complex (320 000 c.p.m./200 μ g of protein per 200 μ l of 0.9% NaCl) was injected into the portal vein of rats under Nembutal anaesthesia. They were killed after 5 min, livers dissected and used for isolation of parenchymal cells. The cell-associated radioactivity was determined to assess the uptake of the complex.

Isolation of hepatic parenchymal cells

The method of Howard & Pesch (1968) was used, which consisted of dissecting out the entire liver and perfusing it through the portal vein with 15 ml of ice-cold Ca^{2+} -free Hanks solution containing 0.05% collagenase and 0.10% hyaluronidase. Incubation of liver slices with the enzyme solution at 37°C in an atmosphere of O_2/CO_2 (19:1) for 70 min was followed by filtration through nylon mesh (61 μ m

pore size), centrifugation and resuspension of the cell pellet in Ca^{2+} -free Hanks medium.

Light-microscopic examination of the cells showed they were parenchymal cells with little contamination of cell-free components. Viability of cells assessed using Trypan Blue exclusion indicated that over 80% were viable. The yield of cells was approx. 2.5×10^6 cells/g of liver.

Uptake of avidin-[^3H]biotin in vitro by rat liver parenchymal cells

Rat liver parenchymal cells isolated as above were suspended in Ca^{2+} -free Hanks medium. Cells (0.5×10^6) were incubated at 4°C or 37°C with $100\mu\text{g}$ of avidin-[^3H]biotin complex ($160000\text{ c.p.m./}100\mu\text{l}$ per $100\mu\text{g}$ of protein) for various periods of time up to 30 min. At the end of the incubation the tubes were diluted with 1 ml of ice-cold medium and placed on ice. The cells were washed three times with Hanks medium. The cell pellet was dissolved in NCS by heating for 1 h at 45°C and radioactivity determined by using OCS as scintillant in a liquid-scintillation spectrometer.

Release of cell-associated avidin-[^3H]biotin by acetic acid treatment

The method of Haigler *et al.* (1980) for the removal of cell surface label was used. Rat liver parenchymal cells ($1 \times 10^6/0.9\text{ ml}$ of Ca^{2+} -free Hanks medium) were incubated with $100\mu\text{l}$ of avidin-[^3H]biotin complex ($160000\text{ c.p.m./}100\mu\text{g}$ of protein) for 30 min at 4°C and 37°C respectively. At the end of this the cells were washed five times each with 1 ml of Hanks medium. After this, half the incubated tubes were used for acid treatment and the other half used for determining total cell-associated radioactivity. The tubes were treated with 1 ml of 0.2 M-acetic acid containing 0.5 M-NaCl for 6 min at 4°C . The acid extract and one additional wash with 0.5 ml of acid were pooled. Radioactivity associated with the pellets and acid extracts was determined.

Preparation of plasma membrane fraction

Rat livers were homogenized by the method of Neville (1968) and plasma membranes were prepared by the method of Yunghans & Morré (1973). Rat intestinal mucosa plasma membranes were prepared by the method of Kessler *et al.* (1978) from the first 20 cm length of small intestine. Electron microscopy of the membrane fractions indicated the absence of nuclei, mitochondria, lysosomes, rough endoplasmic reticulum and Golgi bodies. Enrichment of membrane fractions was determined by using the activity of marker enzymes. The activity of 5'-nucleotidase (EC 3.1.3.5) was assayed by the method of Morré (1977) and the activity of alkaline phosphatase (*p*-nitrophenyl phosphatase; EC 3.1.3.1) by the method of Lowry *et al.* (1954).

Evidence for the location of alkaline phosphatase in rat liver plasma membrane has been provided by Emmelot & Bos (1966) and Lansing *et al.* (1967). The use of alkaline phosphatase as one of the markers for small-intestine brush-border membrane has been described by Kessler *et al.* (1978). Using alkaline phosphatase as marker, liver plasma membrane was enriched 20.3-fold and intestinal mucosa plasma membrane 15.5-fold. Examination of the membrane preparations for acetyl-CoA carboxylase (EC 6.4.1.2) by the method of Dakshinamurti & Desjardins (1969) and propionyl-CoA carboxylase (EC 6.4.1.3) by the method of Hsia *et al.* (1979) demonstrated the absence of both enzyme activities. Protein was determined by the method of Peterson (1977).

Preparation of the avidin derivatives

The avidin-[^3H]biotin was prepared by the method of Dakshinamurti & Chalifour (1981). [^3H]Avidin was prepared by the method of Tack *et al.* (1980) with a specific radioactivity of 347 Ci/nmol. The ability to bind biotin was retained after ^3H labelling.

Binding assay

Plasma membrane ($30\mu\text{g}$ of membrane protein) was added to initiate binding in 1.5 ml Eppendorf centrifuge tubes containing 3 mg of bovine serum albumin, Earle's balanced salt solution, pH 6.0, and avidin-[^3H]biotin complex ($4\mu\text{g}$ of protein), in a total reaction volume of $300\mu\text{l}$. The binding was allowed to proceed for 30 min at room temperature. The samples were then diluted to 1.3 ml with Earle's balanced salt solution and centrifuged for 10 min at 4°C in an Eppendorf microfuge. The supernatant was removed by aspiration and the samples were washed with 1 ml of Earle's balanced salt solution and recentrifuged. The second supernatant was removed by aspiration, $100\mu\text{l}$ of Earle's balanced salt solution was added and the contents of the Eppendorf tube were sonicated for 10 min at 4°C . The sonicated material was quantitatively transferred to a scintillation vial and solubilized by NCS. OCS scintillator was added and radioactivity determined in a Beckman LS-50 scintillation spectrometer after dark damping. Specific binding is defined as the difference between total and non-specific binding of avidin-[^3H]biotin to plasma membrane. Total binding was that in the absence of unlabelled avidin, whereas non-specific binding was that observed when excess unlabelled avidin was added to the binding assay. Parallel sets of tubes either with or without excess unlabelled avidin were included in the incubations to determine specific binding. Scatchard plots of the specific binding data were used to determine K_d and B_{max} values (Scatchard, 1949). In all experiments, except as noted, specific binding was determined.

Results

Uptake in vivo of avidin-[³H]biotin by rat liver cells

The avidin-biotin complex was injected directly into the portal veins to avoid dilution before uptake by the liver. To ensure that only cellular uptake rather than radioactivity associated with blood circulating in the liver was determined, we isolated liver parenchymal cells to determine the true uptake. The isolated liver parenchymal cells retained 31% of the injected radioactivity, thus indicating that parenterally administered avidin-biotin complex is taken up by liver cells under conditions *in vivo*.

Uptake of avidin-[³H]biotin in vitro by rat liver parenchymal cells: effect of temperature

The uptake of avidin-[³H]biotin complex by liver parenchymal cells has been plotted as a function of time and temperature in Fig. 1. It was noted that at 4°C a significant amount of the label was associated with the cells but this did not increase considerably with time indicating adsorption of the label to the cell. The uptake was temperature dependent and reached a plateau 15 to 20 min after a rapid initial uptake.

Fig. 1. Effect of temperature on the uptake in vitro of avidin-[³H]biotin complex by rat liver parenchymal cells

Liver cells suspended in Ca²⁺-free Hanks medium were incubated with avidin-[³H]biotin (160000 c.p.m./100 µg of protein per 100 µl) for various periods of time at 37 and 4°C respectively. Radioactivity of the washed cell pellets was determined.

Internalization of the avidin-[³H]biotin complex

To determine if the cell-associated label was surface-bound or internalized we applied the criteria of acetic acid removal of cell surface label as used by Haigler *et al.* (1980). When liver cells were incubated with avidin-biotin complex at 4°C for 30 min and then treated with 0.2M-acetic acid at 4°C for 6 min 74% of the total label (cell pellet plus acid extract) was present in the acid extract and only 26% remained in the pellet. However, when the cells were incubated with avidin-biotin complex at 37°C for 30 min and then treated with acetic acid only 32% of the total label could be extracted with acid, whereas 68% was associated with the pellet.

Binding of avidin-[³H]biotin complex to rat liver plasma membrane

The binding of avidin-[³H]biotin to liver plasma membrane is shown in Fig. 2(a). It is temperature-dependent. At 37°C binding reached a plateau after 20 min of incubation and at room temperature after 30 min. Total binding at 18°C was only 25% of that at 37°C. There was only minimal binding at 4°C. The binding of avidin-[³H]biotin to plasma membrane increased linearly with increasing liver plasma-membrane protein concentration. The effect of changing the pH of the incubation and washing medium on binding of avidin-[³H]biotin to liver plasma membrane is shown in Fig. 2(b). The optimal pH for specific binding was less than 6.0. A marked drop was seen between pH 6.0 and 7.0 so that binding at pH 7.0 was only 50% of that seen at pH 6.0. The decreased binding at high pH values might be related to changes in the dissociation of reactive groups on the plasma membranes of avidin.

The effects of increasing concentrations of avidin-[³H]biotin in the presence and absence of excess unlabelled avidin on binding to plasma membrane are shown in Fig. 3(a). Specific binding increased in a concentration-dependent manner and is approx. 60–65% of the total binding. In Fig. 3(b) a Scatchard plot of the specific binding data in Fig. 3(a) is shown. The linearity suggests a single type of binding of the avidin-[³H]biotin to the liver plasma membrane with a K_d of 35 nM and B_{max} of 136 pmol/mg of membrane protein. This K_d value is similar to the K_d of 40 nM reported by Stahl *et al.* (1976) for mannose-BSA, of 43 nM reported by Steer *et al.* (1979) for β -hexosaminidase, of 4 nM for α -N-acetylglucosaminidase and 70 nM for α -mannosidase as reported by Ullrich *et al.* (1978). These macromolecules have all been shown to be internalized by the liver cell when injected into the whole animal (Neufeld & Ashwell, 1980). All the enzymes mentioned above are glycoproteins like avidin (Green, 1975).

Effect of biotin saturation of avidin on specific binding

Each monomer of avidin independently binds a biotin molecule. The effect of the biotin saturation of the avidin on specific binding to the liver plasma membranes was investigated. Based on the known specific activity of biotin, the known biotin-binding capacity of avidin and the protein concentration, the percentage occupancy of biotin binding sites on avidin by [^3H]biotin can be calculated. The saturation of avidin-[^3H]biotin by biotin was varied from 8–100%. In the range of biotin saturation of the avidin molecule studied there was no effect on the binding of the avidin-[^3H]biotin complex to the liver plasma membrane. The site on avidin that binds to the liver plasma membrane presumably is not at or near the biotin binding site. This is in contrast with the binding of avidin-[^3H]biotin to HeLa cells, where binding was reduced by 40% when the percentage saturation of avidin by biotin increased from 8 to 25% (Chalifour & Dakshinamurti, 1982b).

Effect of enzymic pretreatment of plasma membrane on binding of avidin-[^3H]biotin

As the binding of the ligand to liver plasma membrane is mediated by a membrane receptor the effect of various enzyme treatments of the plasma membrane was studied. The effect of trypsin, Pronase and neuraminidase on specific binding of avidin-[^3H]biotin to the liver plasma membrane is presented in Table 1. Treatment of rat liver plasma membrane with 100 μg of Pronase per ml increased the specific binding of avidin-[^3H]biotin complex to 200% of the control value. However, it was sensitive to tryptic hydrolysis as binding dropped to 35% of the control value after exposure to 100 μg of trypsin per ml. Neuraminidase had no effect on binding, indicating that neuraminic acid residues on the liver plasma membrane are not involved in avidin-[^3H]biotin specific binding.

Fig. 2. *Effect of temperature (a) and of pH (b) on binding*

(a) The binding assay medium, minus the liver plasma membrane, was equilibrated for 5 min at 37°C or 18°C. The addition of liver plasma membrane (30 μg of membrane protein) initiated binding and incubation was continued for the time periods indicated after which the membrane-associated radioactivity was determined as described in the text. (b) Earle's balanced salt solution was adjusted to a pH range of between pH 4.0 and 9.0. The pH of the binding assay was the same as that of the medium used to wash the membrane. Liver plasma membrane (30 μg of membrane protein) was incubated for 30 min at 18°C in the binding assay mixture. Membrane-associated radioactivity was determined as described in the text. Points represent individual values.

Fig. 3. Specific binding data (a) and determination of K_d and B_{max} from these data (b) (a) The concentration of avidin- $[^3H]$ biotin in the binding assay was varied from 15 to 150 nM. Parallel incubations with 100 μg of unlabelled avidin (1.5 μM) allowed determination of specific binding at each level of avidin- $[^3H]$ biotin. Liver plasma membrane (30 μg of membrane protein) was added to initiate binding, which was continued for 30 min at 18°C. Membrane-associated radioactivity was determined. x, Avidin- $[^3H]$ biotin alone; •, avidin- $[^3H]$ biotin + unlabelled avidin; o, specific binding of avidin- $[^3H]$ biotin. (b) Specific binding data from (a) were replotted in a Scatchard plot.

Table 1. Effect of enzyme pretreatment of rat liver plasma membranes on avidin- $[^3H]$ biotin specific binding Liver plasma membrane was incubated at 37°C for 20 min respectively with Pronase (10 and 100 μg/ml), trypsin-TPCK (100 and 200 μg/ml) and neuraminidase (12.5 and 25 units/ml) in the binding assay medium minus avidin- $[^3H]$ biotin and bovine serum albumin. Membranes were then washed free of enzyme. Components of the binding assay were added to the membrane preparations and specific binding of avidin- $[^3H]$ biotin was determined. Control membrane preparations were treated identically but were not incubated with enzymes. Membrane-associated radioactivity was determined. Results are means ± s.d. of three determinations.

Enzyme	Specific binding (% of control)
Pronase	
10 g/ml	91 ± 5
100 g/ml	202 ± 13
Trypsin	
100 g/ml	79 ± 6
200 g/ml	35 ± 2
Neuraminidase	
12.5 units/ml	108 ± 8
25 units/ml	103 ± 7

Role of carbohydrate moiety on the binding of avidin-biotin to plasma membrane

The effect of simple carbohydrates and mannan on specific binding of avidin- $[^3H]$ biotin to liver plasma membrane was studied. The results are presented in Table 2. None of the carbohydrates tested inhibited the specific binding. These simple carbohydrates have been demonstrated to inhibit the known carbohydrate specific receptor systems (Neufeld & Ashwell, 1980). It is possible that the absence of secondary structure in simple carbohydrates might be responsible for the lack of inhibition of specific binding. However, mannan that does possess secondary structure also did not inhibit binding. We examined the effect of alteration of the carbohydrate moiety of avidin on its ability to inhibit the binding of avidin- $[^3H]$ biotin to liver plasma membrane. Avidin was treated with periodate by the method of Stahl *et al.* (1976) and with α-D-mannosidase by the method of Baynes & Wold (1976). The periodate- or α-D-mannosidase-treated avidin was able to inhibit avidin- $[^3H]$ biotin binding as well as the untreated avidin in a concentration-dependent manner (Fig. 4). Thus it appeared that alteration or removal of the oligosaccharide chain on avidin did not alter its ability to compete with the binding of avidin- $[^3H]$ biotin complex to plasma membrane.

Table 2. *Effect of carbohydrates on specific binding of avidin-biotin to rat liver plasma membrane*

Avidin-[^3H]biotin complex was incubated for 30 min at 18°C in the binding assay medium containing D-(+)-mannose, D-(+)-glucosamine, D-(+)-glucose, D-(+)-galactose, D-(+)-galactosamine, neuraminic acid, α -L-(-)-fucose, mannose 6-phosphate and yeast mannan respectively at the concentrations indicated. Liver plasma membrane was added to initiate binding and incubated for 30 min at 18°C. Membrane-associated radioactivity was determined. Results are means $\pm$ s.d. of three determinations.

Carbohydrate	Specific binding (% of control)
D-(+)-Mannose	103 $\pm$ 5
D-(+)-Glucose	100 $\pm$ 5
D-(+)-Glucosamine	98 $\pm$ 4
D-(+)-Galactose	95 $\pm$ 2
D-(+)-Galactosamine	90 $\pm$ 3
Neuraminic acid	100 $\pm$ 5
Fucose*	112 $\pm$ 3
Mannose 6-phosphate	105 $\pm$ 8
Mannan†	98.5 $\pm$ 3

* Fucose concentration was 0.1M; all other simple carbohydrates were at 1 mM.

† Mannan concentration was 1 mg/ml.

Fig. 4. *Alteration of the carbohydrate moiety of avidin* Periodate- or α -D-mannosidase-treated avidin and untreated avidin respectively were incubated in increasing concentrations with liver plasma membrane (30 μg of membrane protein) in the binding assay for 30 min at 18°C. Membrane-associated radioactivity was determined. ●, Avidin; ○, avidin, periodate treated; ◇, avidin, mannosidase-treated. Points represent individual values.

Fig. 5. *Lysozyme and ovalbumin competition with avidin-[^3H]biotin binding*

Liver plasma membrane (30 μg of membrane protein) was incubated with increasing concentrations respectively of lysozyme, ovalbumin or unlabelled avidin for 30 min at 18°C in the binding assay with avidin-[^3H]avidin. Membrane-associated radioactivity was determined. Points represent individual values.

Effect of analogous proteins on avidin-[^3H]biotin binding to plasma membrane

It is possible that the basicity of the avidin molecule is significant for the specific binding with plasma membrane. Lysozyme is a very basic protein that has amino acid composition and sequence homologous to avidin (Green, 1975). Hence, the effect of lysozyme on the specific binding of avidin-[^3H]biotin to liver plasma membrane was studied. Ovalbumin has the same high-mannose type of oligosaccharide chain as avidin and so could be expected to inhibit the binding of avidin-[^3H]biotin to liver plasma membrane if both proteins bound to the same receptor. Ovalbumin and lysozyme, as shown in Fig. 5, did not inhibit avidin-[^3H]biotin binding to plasma membrane, whereas unlabelled avidin inhibited this binding in a concentration-dependent manner. Preparation of [^3H]avidin by NaBH_4 reduction results in the methylation of lysine residues (Tack *et al.*, 1980). When the [^3H]avidin was substituted for avidin-[^3H]biotin no specific

binding was observed, indicating that the methylation of the lysine residues interfered with specific binding.

Binding of avidin-[³H]biotin complex to rat intestinal mucosa plasma membrane

It has long been assumed that the reason orally administered avidin or dried raw egg white, which contains avidin, produced biotin deficiency in higher organisms is because the avidin-biotin complex is not digested and absorbed in the gastrointestinal tract. Cells like the HeLa and human fibroblasts can internalize the avidin-biotin complex (Chalifour & Dakshinamurti, 1982a). The ability of intestinal mucosa cells to internalize macromolecular complex containing vitamin B₁₂ is well recognized (Rosenberg *et al.*, 1975). In view of this, we examined the ability of intestinal mucosa cells to take up the avidin-biotin complex. As the first step in such a procedure would be the binding of the avidin-biotin complex to the plasma membrane of these cells the binding of the avidin-[³H]biotin complex to the isolated intestinal mucosa plasma membrane was examined. The rat intestinal mucosa plasma membrane, when incubated in the binding assay system developed for rat liver plasma membrane, did not bind avidin-[³H]biotin at high (200 µg) membrane protein concentrations or at high (300 nM) avidin-[³H]biotin concentrations.

Discussion

After intraportal administration of avidin-biotin complex a substantial portion of the label was associated with liver parenchymal cells. Thus the uptake is an '*in vivo* phenomenon' and hence of significance. The uptake *in vitro* of avidin-biotin complex by isolated liver parenchymal cells was temperature-dependent. Although substantial label is associated with the cell at 4°C there was little uptake with time, indicating adsorption of the label to the cell surface. This was confirmed by the observation that 74% of the cell-associated radioactivity was acid-extractable. Internalization of the avidin-biotin complex at 37°C is indicated by the fact that only 32% of the cell-associated radioactivity was acid-extractable, whereas 68% was associated with the cell pellet. These observations also indicate that avidin-biotin complex binds to the external surface of liver parenchymal cells before the temperature-dependent internalization, a situation akin to that reported for the HeLa cells and human fibroblasts (Chalifour & Dakshinamurti, 1982a,b).

The investigation of the binding of the avidin-[³H]biotin complex to the rat liver plasma membrane was initiated to determine if specific binding occurred and to characterize this binding. The binding of the complex was found to be tem-

perature- and pH-dependent, saturable and specific. The receptor was exposed by Pronase treatment but sensitive to tryptic hydrolysis and insensitive to neuraminidase. Specific binding had a K_d of about 35 nM and a B_{max} of about 140 nM. Binding was not directed by the oligosaccharide chain of avidin as indicated by the lack of inhibition of binding by simple and complex carbohydrate structures. Avidin does not seem to bind to liver plasma membrane at or near the biotin binding site. Lysozyme, a very basic protein similar to avidin, did not inhibit [³H]biotin binding, suggesting that the position rather than the number of basic residues is important in the specific binding. Modification of lysine residues of avidin has been found to abolish the specific binding of avidin to liver plasma membrane. Similar results have been obtained for other proteins. Modification of lysine and arginine residues resulted in decreased binding of low-density lipoprotein (Kita *et al.*, 1981; Pangburn *et al.*, 1981) and ribonuclease (Kooistra *et al.*, 1979), whereas modifications of tyrosine and histidine were without any effect (Aviram *et al.*, 1981). Avidin-biotin complex did not bind to intestinal mucosa plasma membranes. The specific binding of avidin-biotin complex to liver plasma membrane is not through the carbohydrate moiety of avidin and seems to be sensitive to modification of its amino acid residues. As avidin is not a normal circulatory protein it should be mimicking a natural ligand in its specific binding to liver plasma membrane. The identity of this ligand is not known yet. The specificity and high affinity of avidin for biotin has recently been exploited as a tool for cytochemical studies (Bayer & Wilchek, 1980). The evidence presented here foregrounds the importance of appropriate controls in experiments with avidin as a cellular probe.

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