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Interactions of Retinol with Binding Proteins: Implications for the Mechanism of Uptake by Cells†

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ABSTRACT: The kinetic parameters of the interaction of retinol with retinol binding protein (RBP) were studied. The rate constant for association of retinol with the protein (k_a) was found to be 1.5×10^6 M^{-1} min^{-1}. The rate constant for dissociation (k_d) from the protein was determined by studying the transfer of retinol from RBP to lipid bilayers. It was found that such transfer proceeds via the aqueous phase and its rate-limiting step is the dissociation of retinol from the binding protein. The rate of transfer therefore represents the rate of dissociation. The k_d was 0.112 min^{-1}. These values were validated further by the following consideration. The equilibrium dissociation constant of RBP and retinol can be calculated from the expression $K_d = k_d/k_a$. The calculated value was 7.5×10^{-8} M. K_d was also measured directly by fluorometric titration and was found to be 7×10^{-8} M. The relative avidities of retinol for RBP, the complex RBP-transthyretin (RBP-TTR), and serum albumin were also studied. It was found that binding of RBP to TTR increased its avidity for retinol by about 2-fold. The avidity of albumin for retinol was 30-fold lower than that of RBP. The data imply that retinol spontaneously and rapidly dissociates from sites on binding proteins, which indicates that the vitamin can freely move in vivo between physiologic compartments with avidities for it.

Vitamin A alcohol is poorly soluble in water and is transported in blood bound to a protein complex that is constituted of retinol binding protein (RBP) and transthyretin (TTR) (Goodman & Blaner, 1984). The mechanism by which retinol moves from the protein complex in blood to the cytosol of target cells is not clear. This question has been studied by several laboratories, and it is usually proposed that specific receptors for RBP, mediating the uptake of retinol, exist in the plasma membranes of target cells (Chen & Heller, 1977; Rask & Peterson, 1976; McGuire et al., 1981; Heller, 1975; Ottonello & Maraini, 1981; Pfeffer et al., 1986). However, the existence of such receptors has not been demonstrated directly. The evidence given in support of putative receptors is the saturation of uptake of retinol by cells (Chen & Heller, 1977; Rask & Peterson, 1976) or the identification of proteins that bind RBP in plasma membranes (McGuire et al., 1981; Heller, 1975). Neither of these lines of evidence is sufficient

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to prove the existence of a "receptor" (Daniels et al., 1985; Noy et al., 1986; Cooper et al., 1987). Moreover, rates of individual steps of spontaneous transfer of retinol have not been accurately measured but only approximated up till now (Fex & Johannesson, 1987) so that the rate-controlling factors and the rate-limiting step(s) cannot be identified. Consequently, it is not clear what the proposed function of the putative RBP receptors is. Do they participate in uptake by catalyzing the release of vitamin A from RBP to plasma membranes of target cells or by catalyzing movement of the vitamin across these membranes into the cytosol of cells? Alternatively, the putative receptors could function to stabilize the labile vitamin against degradation or to channel it to cytosolic binding proteins for proper function (Pfeffer et al., 1986).

Another protein that binds retinol, at least in vitro, is serum albumin (Futterman & Heller, 1972). The role of albumin in transport of retinol in blood is, however, not clear though it is generally accepted that albumin serves as a transport protein for retinoic acid but not for retinol (Goodman & Blaner, 1984; Goodman, 1984).

An important question connected with the uptake process is then whether any process that delivers retinol to target cells will result in uptake or whether functional uptake of retinol is uniquely dependent on its binding to RBP. The first step in the attempt to decipher the mechanism of uptake of retinol is, therefore, to measure the spontaneous rates of the reactions that lead to transfer of retinol from the binding proteins in serum to the inside of cells. The present work deals with the first phase of the transfer process, namely, with the interactions of retinol with serum proteins with avidity for it.

Materials and Methods

Lipids were obtained from Avanti Polar Lipids. Chromatography gels were purchased from Pharmacia. *all-trans*-Retinol was obtained from Kodak. [^{3}H]Retinol and [^{14}C]-dioleoylphosphatidylcholine were from New England Nuclear. Bovine serum albumin was purchased from Calbiochem. All other chemicals were from Sigma Chemical Co. Scintillation liquid (Liquiscint) was from National Diagnostics. Male Wistar rats weighing about 300 g were obtained from Charles River Breeding Laboratories.

Proteins. Bovine RBP was purified from bovine serum (Pel-Freeze Co.) by the procedure outlined in Berni et al. (1985) and was usually found to contain 0.85–1 mol of retinol/mol of protein. Human RBP and human TTR were a gift from Dr. William S. Blaner. Concentrations of proteins and of retinol were determined from their extinction coefficients (Horowitz & Heller, 1977). Apo-RBP was obtained by extracting retinol from bovine RBP into heptane. Equal volumes of a solution of holo-RBP and heptane were mixed gently at room temperature for 4 h. Over 90% of bound retinol was removed by this procedure as judged by the ratio of absorbance at 280 and 330 nm.

Membranes. Small unilamellar vesicles were prepared by sonication. The appropriate lipid in chloroform or hexane solution was pipetted into a stainless-steel cup and the organic solvent evaporated under a stream of argon or nitrogen. Buffer containing 100 mM NaCl, 10 mM HEPES (pH 7.0), and 1 mM ascorbate was added and the suspension sonicated in a Heat-System sonicator to clarity. Vesicles were centrifuged at 100000*g* for 15 min to pellet multilamellar vesicles. The concentration of lipids was determined by the phosphorus content (Dittmer & Wells, 1969). Plasma membranes were isolated from rat liver on density gradients (Song et al., 1969).

Dissociation Constant of Retinol from Bovine RBP. This was measured by fluorometric titration as described by Cogan et al. (1976). Apo-RBP was titrated with retinol dissolved in ethanol, and the titration was followed by measuring the fluorescence of the RBP–retinol complex (excitation, 290 nm; emission, 480 nm). The mass law equation

$$K_d = nPR/PR \quad (1)$$

was used to derive a working equation for the evaluation of the apparent dissociation constant (K_d). In eq 1, P, R, and PR are the concentrations of free RBP, free retinol, and retinol bound to RBP, respectively. n is the number of binding sites for retinol per mole of RBP. If α is defined as the fraction of free binding sites on the protein molecule and P_0 and R_0 are the total protein and retinol concentrations, respectively, eq 1 can be written in the form of eq 2. A plot of $P_0\alpha$ vs

$$P_0\alpha = (1/n)[R_0\alpha/(1 - \alpha)] - K_d/n \quad (2)$$

$R_0\alpha/(1 - \alpha)$ will have a slope of $1/n$ and an intercept of K_d/n. The value of α was calculated for every point on the titration curve of fluorescence intensity vs total retinol concentration by using the relation:

$$\alpha = (F_{max} - F)/(F_{max} - F_0) \quad (3)$$

where F_0, F, and F_{max} are the fluorescence intensities at R_0, at R, and at saturation, respectively. Calculations and corrections were performed as in Cogan et al. (1976).

Rate of Association of Retinol with RBP. One nanomole of apo-RBP in 1 mL of buffer containing 20 mM potassium phosphate and 200 mM NaCl was placed in a cuvette. One nanomole of retinol dissolved in 1 mM Triton X-100 was added, and the fluorescence (excitation, 330 nm; emission, 480 nm) was followed at 0.5-min intervals until equilibrium was reached.

Rate of Transfer of Retinol between RBP and Vesicles. A mixture of 0.6–1 nmol of RBP containing 0.85–1 mol of retinol/mol of protein was mixed in a cuvette with vesicles of egg phosphatidylcholine in a buffer containing 20 mM potassium phosphate and 200 mM NaCl. The final volume was 1 mL. Fluorescence (excitation, 290 nm; emission, 480 nm) was followed at 1-min intervals until equilibrium was reached. The mixture was kept in the dark between measurements to minimize photodegradation of retinol.

Distribution of Retinol between Binding Proteins and Plasma Membranes of Rat Liver Cells. RBP was mixed with a trace amount of [^{3}H]retinol to give a specific radioactivity for retinol of about 40000 cpm/nmol. The mixture was incubated at 4 °C in the dark for 1 h to complex the ligand with the protein. The labeled protein (4–6 nmol of protein) was then mixed with plasma membranes (40–60 nmol of phospholipids) in a final volume of 0.5 mL. The mixture was incubated for 45 min at 20 °C and then centrifuged in an Eppendorf centrifuge for 30 min; 0.2 mL of the supernatant containing the protein was counted for [^{3}H]retinol. The rest of the supernatant was decanted, and the bottom of the Eppendorf tube (i.e., the plasma membranes pellet) was cut, dropped into a scintillation vial, and counted for retinol. Distribution constants were expressed as the ratio of the mole fractions of retinol associated with the protein and the lipids. To correct for the amount of retinol in the supernatant that is dissolved in the aqueous phase, the distribution constant of retinol between the plasma membranes and water was measured. This was done by the same procedure but without the protein. The results were corrected according to that constant which was found to be 0.6×10^6 (molal fraction).

Results

Binding Affinity of Retinol to RBP. In the simplest potential spontaneous process of transfer of retinol to cells, the

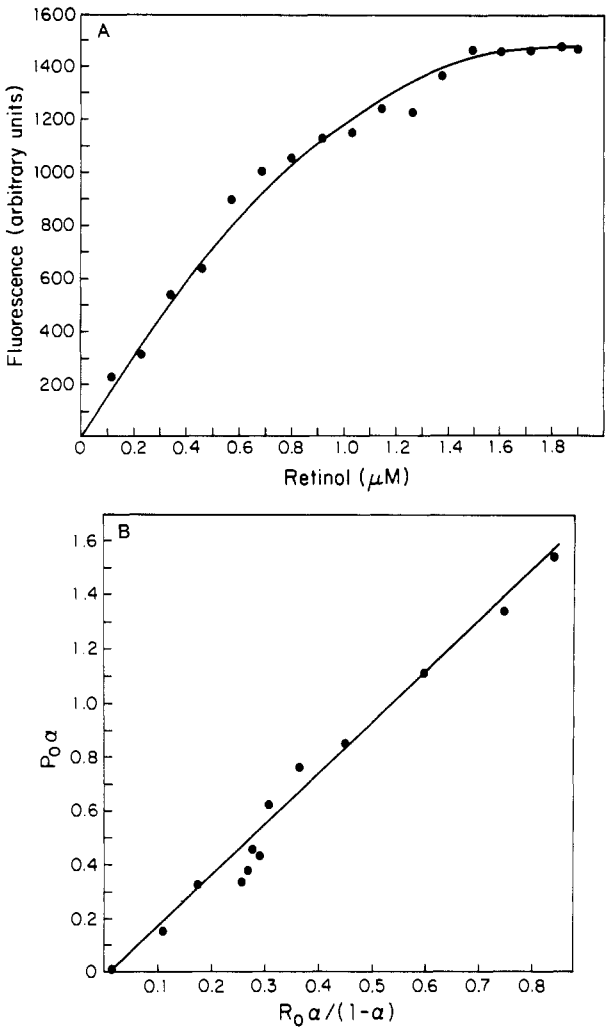

FIGURE 1: (A) Fluorometric titration of apo-RBP with retinol. The titration system consisted of 1 mL of 1.96 μM bovine apo-RBP in 20 mM potassium phosphate, pH 7.0, and 150 mM NaCl. Retinol was added from a solution in ethanol. Most of the data were obtained by using 1–6 μL of ethanol. The maximal volume of ethanol added was 17 μL. Excitation wavelength, 290 nm; emission wavelength, 480 nm. Data shown are corrected (See Materials and Methods). (B) Plot of eq 2, $P_0\alpha$ vs $R_0\alpha/(1-\alpha)$, for the titration of apo-RBP with retinol shown in (A). See Materials and Methods for explanation.

first step would be the dissociation of retinol from RBP to the aqueous phase as described by reaction I. The dissociation constant is $K_d = k_1/k_{-1}$.

reaction I

$$\text{RBP–ret} + n(H_2O) \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \text{RBP} + \text{ret–}(H_2O)_n$$

The dissociation constant of retinol from bovine RBP was measured by fluorometric titration utilizing the energy transfer that is known to exist between tryptophanyl residues which are located in the vicinity of the binding site on RBP and bound retinol (Goodman & Leslie, 1972). Thus, excitation of the RBP–retinol complex at 290 nm results in fluorescence at the emission band of retinol (around 480 nm). A typical titration of the protein with retinol gives the binding curve shown in Figure 1A. Analysis of the data according to eq 2 (see Materials and Methods) is shown in Figure 1B. From the intercept and the slope of the straight line in Figure 1B, the apparent dissociation constant and the number of binding sites can be calculated. These are 0.7×10^{-7} M (SEM = 0.22×10^{-7} M, $n = 4$) and 0.8 mol of retinol/mol of protein, respectively. The reported value of K_d for both human RBP and

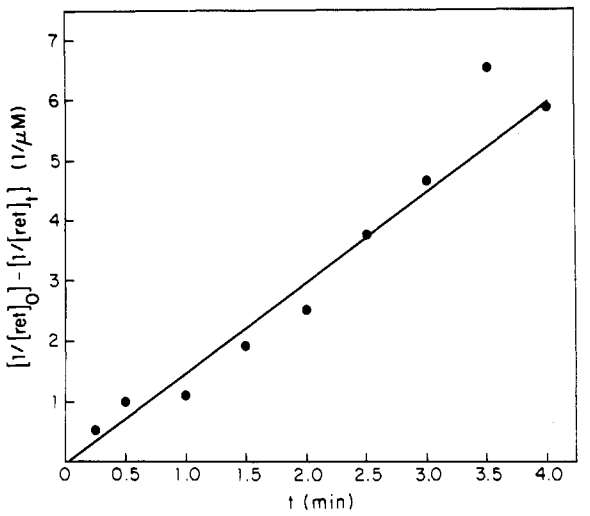

FIGURE 2: Association of retinol with RBP. One nanomole of RBP was placed in a cuvette. The final volume was 1 mL, and the buffer contained 20 mM potassium phosphate, pH 7.0, and 150 mM NaCl. Ten microliters of a 0.1 mM suspension of retinol in 1 mM Triton X-100 was added, and the fluorescence intensity (excitation, 340 nm; emission, 480 nm) was followed until equilibrium was reached. The data were plotted as $1/[ret]_0 - 1/[ret]_t$. The slope of the plot is k_{-1}, the rate constant of the association reaction (see text).

chicken RBP is 1.5×10^{-7} M (Cogan et al., 1976; Muhilal & Glover, 1975).

Rate of Association of Retinol with RBP. The rate constant of association for retinol with RBP (k_{-1}) was measured by utilizing the enhancement of retinol fluorescence upon binding (Cogan et al., 1976). Retinol was suspended in 1 mM Triton X-100 and mixed with RBP. The fluorescence of the mixture (excitation, 340 nm; emission, 480 nm) was followed at 0.5-min intervals until equilibration. Since the concentrations of both RBP and retinol at $t = 0$ in this experimental setup were the same and since the stoichiometry of binding is 1:1, the second-order rate constant can be obtained by plotting $1/[ret]_0 - 1/[ret]_t$ vs t as is shown in Figure 2. The value of k_{-1} thus obtained was $(1.5 \pm 0.3) \times 10^6$ M^{-1} min^{-1} ($n = 4$). A potential problem with the experimental setup described is that the measured association constant may represent dissociation of retinol from micelles of the detergent used to suspend it. To test for this, the rate of association was measured as well by adding retinol from a solution in ethanol. The results were essentially the same (data not shown). It should be pointed out, in this regard, that the system is inherently difficult because retinol forms micelles in an aqueous solution even at very low concentrations (Radda & Smith, 1970). Consequently, for a monomer of retinol to associate with a binding protein, it must first dissociate from the micelle. It seems, however, that the rate of association with the protein is the rate-limiting step in the system(s) described above and that the apparent rate constant measured is indeed k_{-1}. This can be inferred from the identical value obtained when retinol was initially dissolved in Triton X-100 or in ethanol. Thus, if desorption of retinol from the micelle was the rate-limiting step, it is unlikely that desorption from the mixed detergent/retinol micelles will proceed at the same rate as desorption from the pure retinol micelles. This conclusion was validated further by comparing the value of the equilibrium dissociation constant calculated from the expression $K_d = k_1/k_{-1}$ with the value of K_d obtained by direct measurement (see below).

Rate of Dissociation of Retinol from RBP. Since retinol is poorly soluble in water, it is difficult to induce its dissociation from RBP in accurately measurable amounts in a system

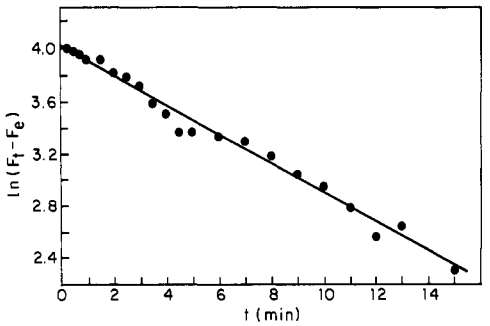

FIGURE 3: Transfer of retinol from RBP to unilamellar vesicles of egg phosphatidylcholine. Holo-RBP (1.38 μM) containing 0.85 mol of retinol/mol of protein in 1 mL of buffer containing 20 mM potassium phosphate and 200 mM NaCl was mixed with egg phosphatidylcholine vesicles (60 μM). Transfer of retinol from the protein to the lipids was followed by monitoring the decrease in the fluorescence of retinol (emission, 470 nm) upon excitation of RBP (280 nm). The data were plotted as ln ($F_t - F_e$) vs time. F_t and F_e denote the fluorescence at time t and at equilibrium, respectively.

consisting of RBP, retinol, and water alone. For this end, another component with high avidity for retinol needs to be added in order to induce dissociation of an amount of retinol that is large enough to monitor the reaction accurately. The component chosen for this purpose is unilamellar vesicles of phospholipids. This was chosen because of the high solubility of retinol in lipids, because vesicles are not expected to directly interact with RBP, and because they closely resemble biological membranes. Dissociation of retinol from RBP was thus followed after mixing holoprotein with small unilamellar vesicles of egg phosphatidylcholine. Enhancement of retinol fluorescence occurs upon its binding to both RBP and lipid bilayers; this method could not be used, therefore, to monitor transfer from protein to bilayers. Instead, the energy transfer between RBP and bound retinol was utilized. The rate of decrease in fluorescence upon mixing of holo-RBP with phospholipid vesicles was used directly to determine the rate of transfer to retinol from the protein to the vesicles. Holo-RBP containing 0.9 mol of retinol per mole of protein was mixed with vesicles, and the fluorescence (excitation, 290 nm; emission, 480 nm) was monitored at intervals up to 60 min. Figure 3 shows the results of a typical time course plotted as ln ($F_t - F_e$) vs t (F_t denotes the fluorescence at time t and F_e is the final value at equilibrium). The transfer process is apparently a single first-order reaction with a rate constant $k_1 = 0.112 \pm 0.015$ min^{-1} ($n = 6$).

The simplest potential transfer process would include, as the first step, the dissociation of retinol from the protein into the aqueous phase (reaction I). Retinol will then diffuse to a bilayer and bind to it as is described by reaction II. Alternatively, retinol could be directly transferred from protein to bilayer during collisions as in reaction III.

reaction II

$$\text{ret}-(H_2O)_n + \text{bilayer} \rightarrow \text{bilayer}-\text{ret} + n(H_2O)$$

reaction III

$$\text{RBP}-\text{ret} + \text{bilayer} \rightleftharpoons \text{bilayer}-\text{ret} + \text{RBP}$$

To clarify the mechanism of spontaneous transfer from RBP to lipid bilayers, the rate of transfer was measured at various protein/lipid mole ratios. If transfer occurred during collisions, then increasing the concentration of the acceptor (lipid bilayer) phase should result in an increased rate of transfer. If, on the other hand, transfer proceeds via the water, the rate-limiting step would be the dissociation from RBP and the transfer rate would be independent of the donor/acceptor concentration ratio. The data (Table I) show that transfer from RBP proceeds with the same rate constant even though the donor/acceptor ratio was varied by 10-fold, indicating that transfer occurs via reactions I and II.

Table I: Rate Constants for Transfer of Retinol from RBP to Egg Phosphatidylcholine Vesicles at Various Lipid/Protein Ratios[a]

lipid/protein ratio (mol/mol)	k (min^{-1})	$t_{1/2}$ (min)
43.5	0.113	6.13
174.0	0.133	5.20
263.4	0.120	5.73
435.0	0.080	8.60

[a] Rates of transfer were measured as described under Materials and Methods. Holo-RBP was mixed with varying amounts of unilamellar vesicles of dioleoylphosphatidylcholine to give the designated lipid/protein mole ratios. Data were collected and analyzed as described in Figure 3.

The slowest step of the transfer process is, most likely, the dissociation of retinol from RBP. Subsequent steps are diffusion to the bilayer and association with it, which are rapid [e.g., see Noy et al. (1986), Lange et al. (1983), Storch and Kleinfeld (1986), and Doody et al. (1980)]. The observed rate constant for transfer represents therefore the rate constant for dissociation, k_1.

The value of the equilibrium dissociation constant of retinol and RBP can be calculated from the relation $K_d = k_1/k_{-1}$. The calculated value is $K_d = 0.75 \times 10^{-7}$ M, which is very similar to the value obtained in the fluorometric titration.

Binding Avidity of Serum Albumin for Retinol Relative to RBP. Serum albumin transports in blood many metabolites that are poorly soluble in water (Spector & Fletcher, 1978; Kamisaka et al., 1974). Albumin binds retinol in vitro, but it is accepted dogma that in vivo retinol is bound solely to RBP (Goodman & Blaner 1984; Goodman, 1984). However, the data presented above indicate that retinol dissociates from RBP fairly rapidly, an observation that implies that retinol distributes between RBP and albumin in blood. It is possible that the distribution of retinol into albumin is not apparent because large differences in avidity of binding effectively exclude retinol from binding to albumin in blood. To test this, the relative avidities of the two binding proteins for retinol were measured. This was not measured directly because of the difficulty of separating the proteins without disturbing the distribution of retinol between them. The distribution of retinol was measured, therefore, between RBP or albumin and plasma membranes of hepatocytes that are easily separated from the proteins by centrifugation. The relative avidities of the proteins can then be calculated by comparing their avidities relative to the plasma membranes. The distribution constants of retinol between the specific proteins and the membranes (K_{eq}) were calculated as K_{eq} = [mol of $(\text{ret})_p$/mol of protein]/[mol of $(\text{ret})_m$/mol of lipid] where $(\text{ret})_p$ and $(\text{ret})_m$ denote the amount of retinol associated with the protein and with the membranes, respectively. K_{eq} for RBP was found to be 25.1 ± 4 ($n = 3$); K_{eq} for albumin was 1.1 (average of two experiments). The avidity of bovine RBP for retinol is about 30-fold higher than that of bovine serum albumin. The concentration of albumin in blood is, however, 500 μM, while the concentration of RBP is 2 μM (Goodman & Blaner, 1984; Spector & Fletcher, 1978); the concentration of albumin is thus 250-fold higher than that of RBP. The observed differences in avidity, therefore, do not account for lack of distribution of retinol into albumin in vivo.

In blood, RBP is bound to TTR, and it was reported that the binding avidity of retinol to the RBP–TTR complex is

somewhat higher than to RBP alone (Fex & Johannesson, 1987). The distribution constants of retinol between human RBP or RBP–TTR and plasma membranes were measured. K_{eq} for human RBP was essentially the same as for the bovine protein; K_{eq} for the RBP–TTR complex was 43.2 (average of two experiments), showing an increase of about 2-fold in the avidity of the complex for retinol as compared to RBP alone.

Another factor that may affect the avidity of albumin for retinol in vivo is the presence of other albumin-bound ligands. The only such ligands that are present in large enough amounts to saturate a binding site on albumin are free fatty acids that are normally found in blood bound to albumin at a mole ratio of 0.5–1 (Spector & Fletcher, 1978). The distribution constant of retinol between albumin and plasma membranes was measured in the presence of palmitate at a palmitate/albumin mole ratio of 1 and was found to be 2.5. The data, then, show a slight increase in the avidity of albumin for retinol when palmitate is bound to the protein. It is apparent that retinol does not compete with free fatty acids on the same binding site on albumin.

Discussion

The results of the present work point to the conclusion that the retinol carrier system is a dynamic one in which retinol can spontaneously move between the various physiologic compartments with avidity for it, i.e., binding proteins and lipid bilayers. For example, the data reported above indicate that retinol spontaneously dissociates from RBP at a rate that is quite rapid (k_1 is 0.123 min^{-1}). Qualitatively similar findings have been recently reported (Fex & Johannesson, 1987) showing spontaneous transfer of retinol from RBP to lipid vesicles. A crude estimate of the rate of transfer was given stating that equilibrium was reached within 60 min (Fex & Johannesson, 1987). Similar findings showing rapid dissociation of retinol from another binding protein (the interphotoreceptor RBP) were also reported (Ho et al., 1989). Other reports indicated rapid movement of retinol between lipid bilayers and membranes [e.g., see Rando and Bangerter (1982) and Fex and Johannesson (1988)].

It is interesting to note, with regard to the data presented here, the recently reported rates of uptake by placental brush border membrane vesicles of retinol initially bound to human RBP (Sivaprasadarao & Findlay, 1988). Uptake was found to follow a first-order kinetic pattern that had a rate constant of 0.2 min^{-1}. The findings in that paper were interpreted by its authors to show that uptake is a receptor-mediated process. However, the reported rate constant for uptake is very similar to the k_1 reported here. Moreover, it was found in the above quoted study that uptake from the complex TTR–RBP had a rate constant that was about 2-fold slower than uptake from RBP alone (Sivaprasadarao & Findlay, 1988). This value closely corresponds to the difference found here between the avidities of RBP and to TTR–RBP for retinol which most likely reflects the difference in the rate of dissociation of retinol from RBP and from RBP when bound to TTR. The results of the above quoted study seem, therefore, to indicate that the rate-determining step of the uptake into placental membrane vesicles was reaction I, i.e., the dissociation of retinol from RBP.

To determine whether the rate of dissociation of retinol from RBP is rapid enough to account for transfer of retinol from blood to cells, or whether transfer in vivo is facilitated, actual rates of uptake by cells should be compared to the rate of the spontaneous dissociation. Few studies have reported rates of uptake of retinol by cells: Rask et al. (1980) reported a rate of uptake by corneal epithelial cells of about 2×10^{-3} nmol of retinol/min in a mixture that included 1 nmol of holo-RBP. If the dissociation of retinol from RBP was the rate-limiting step, the rate of uptake can be calculated by using k_1 obtained in the present study and the following expression: uptake rate = k_1[holo-RBP]. This calculation gives a value of 0.2 nmol/min, which is a 100-fold faster than the observed rate, indicating that reaction I was not rate limiting for uptake. In another study, Rask and Peterson (1976) measured the rate of uptake of retinol by mucosal epithelial cells from small intestine. In one of those experiments, there was 7 nmol of RBP [see Figure 5 in Rask and Peterson (1976)]. The expected rate of uptake, if dissociation from RBP was the rate-limiting step, would be 1.4 nmol/min. The observed rate of uptake in that experiment was, however, 5×10^{-3} nmol/min, again pointing to the conclusion that reaction I was not rate limiting for uptake by these cells.

Some of the evidence published to support the idea that uptake of retinol is mediated by a receptor in the plasma membranes of cells is based on observations showing competition for uptake between RBP-bound, radiolabeled retinol and RBP-bound, unlabeled retinol (Ottonello et al., 1987; Pfeffer et al., 1986). Clearly, this line of evidence is only valid if dissociation of retinol from RBP was the rate-limiting step for uptake. This, as noted above, has not been proven as yet.

An interesting question that is raised by the data of the present work is that we could not find any reason why retinol would not be associated with albumin in blood. Thus, it was found that the avidity of albumin for retinol is about 60-fold lower than the avidity of the TTR–RBP complex. However, the concentration of albumin in blood is about 250-fold higher than that of the latter protein complex. Thus, if retinol is equilibrated between these proteins, a large fraction of it should be found associated with albumin, contrary to what is usually believed. A possible explanation is that retinol is not usually found bound to albumin because methods of isolation of serum albumin result in loss of that ligand, or that binding to albumin does not stabilize retinol as well as binding to RBP (Futterman & Heller, 1972) so that the vitamin degrades during isolation.

The question whether binding to RBP is essential for functional delivery of retinol to target cells remains, but the results presented above seem to put this question in a different perspective: if a unique pathway is physiologically required for entry of retinol to cells, how would rapid spontaneous entry be prevented?

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Kinetic Parameters of the Interactions of Retinol with Lipid Bilayers†

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ABSTRACT: The process of transfer of vitamin A alcohol (retinol) between unilamellar vesicles of phosphatidylcholine was studied. The transfer was found to proceed spontaneously by hydration from the bilayer and diffusion through the aqueous phase. The rate-limiting step for transfer was the dissociation from the bilayer, a step that was characterized in bilayers of egg phosphatidylcholine (PC) by a rate constant k_{off} = 0.64 s^{-1}. The rate constant for association of retinol with bilayers of egg PC was also determined: k_{on} = 2.9 × 10^6 s^{-1}. The relative avidities for retinol of vesicles comprised of PC lipids with the various fatty acyl chains were measured. It was found that the binding affinity was determined by the composition of the lipids, such that PC with symmetric acyl chains had a lower affinity for retinol vs those with mixed chains. To clarify the mechanism underlying this observation, the rates of dissociation and association of retinol bound to vesicles of dioleoyl-PC were determined. The rate of association of retinol with bilayers strongly depended on the composition of the fatty acyl chains of the lipids. The rate of dissociation of retinol from the bilayers of PC was found to be independent of that composition. The implications of the observations for the interactions of hydrophobic ligands with lipid bilayers are discussed.

Retinol (vitamin A alcohol), which is poorly soluble in water, circulates in blood bound to a water-soluble transport protein (Goodman, 1984). To explain how it is transported from blood to cells, it is usually proposed that the vitamin moves directly between several types of carrier proteins. Thus, it is hypothesized that transfer of retinol from serum retinol binding protein (RBP) to cells is mediated by specific receptors that are imbedded in plasma membranes (Chen & Heller, 1977; Rask & Peterson, 1976; McGuire et al., 1981; Heller, 1975; Ottonello & Maraini, 1981; Pfeffer et al., 1986) and that retinol transfers from these receptors directly to a cellular retinol binding protein (CRBP). CRBP, in turn, is proposed to be involved in carrying the vitamin to its sites of action (Chytil & Ong, 1984; Pfeffer et al., 1986).

It has been recently shown, however, that retinol spontaneously dissociates from RBP in vitro in model systems that do not contain any receptors (Fex & Johannesson, 1987; Noy & Xu, 1990). Free retinol will rapidly associate with lipid bilayers or with membranes if these components are present. Recent reports also indicate that retinol readily transfers across and between phospholipid bilayers (Fex & Johannesson, 1988; Noy, 1987, 1988). It is difficult to see, therefore, how association of retinol with the lipids of biological membranes and its movement between them can be prevented in vivo. The solubility of retinol in biological membranes then may have significant consequences for the mechanism by which this compound is distributed between different cells and different cellular compartments.

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