Biochimica et Biophysica Acta 898 (1987) 187–195
Elsevier

BBA 73511

Transport of Fe^{2+} across lipid bilayers: possible role of free fatty acids

Robert J. Simpson and Timothy J. Peters

Division of Clinical Cell Biology, MRC Clinical Research Centre, Watford Road, Harrow, Middlesex HA1 3UJ (U.K.)

(Received 19 August 1986)

Key words: Free fatty acid; Iron transport; Liposome; Brush-border membrane; (Rabbit intestine)

Fatty acids can form lipid-soluble complexes with Fe^{2+}. Incorporation of fatty acids into phosphatidylcholine/cholesterol liposomes renders them permeable to Fe^{2+}. Of several fatty acids tested, the most effective Fe^{2+} carriers were linoleic and oleic acids followed, in decreasing order of efficacy, by linolenic, myristic, arachidonic and palmitic acids. The initial Fe^{2+} transport rate for oleic acid depends on free Fe^{2+} in the medium which in turn shows a strong pH dependence above pH 7.0. The overall pH dependence of Fe^{2+} transport for several fatty acids shows an optimum near 6.9. Fe^{2+} transport catalysed by oleic acid can be inhibited by high NaCl concentrations but not 1 mM Co^{2+}. It is suggested that free fatty acids may act as mediators of Fe^{2+} transport across biological membranes, particularly isolated intestinal brush-border membrane.

Introduction

Artificial phospholipid bilayers and certain natural membranes (e.g. erythrocyte membrane) are relatively impermeable to iron [1]. It is thought that specific cellular membranes allow passage of iron across them but the molecular mechanisms of iron translocation across membranes are obscure, except in the case of the high-affinity siderophore-mediated scavenging mechanisms which operate in microorganisms [2]. In higher animals, iron must cross membranes during iron absorption and during intracellular iron mobilization and reticulocyte development, although the mechanisms of membrane transport are obscure.

We have been studying iron transport across intestinal brush-border membrane, the first step in iron absorption [3,4]. Recently, membrane lipids have been implicated as a possible membrane carrier in brush-border membrane vesicles [4]. Isolated brush-border membranes have been reported by several groups to have high levels of non-esterified fatty acids (3–10% of total lipid), in spite of the use of very different techniques of membrane isolation [5–7]. In contrast, the Fe^{2+} impermeable erythrocyte membrane has extremely low free fatty acid levels (less than 0.15% of total lipids [8]). We therefore set out to investigate the effect of free fatty acids on membrane Fe^{2+} permeability. The dependence of fatty acid-mediated Fe^{2+} transport on several factors (pH, NaCl, Fe^{2+}-ascorbate concentration and Co^{2+}) relevant to brush-border membrane vesicle Fe^{2+} transport are reported.

Abbreviation: Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulphonic acid.

Correspondence: R.J. Simpson, Division of Clinical Cell Biology, MRC Clinical Research Centre, Watford Road, Harrow, Middlesex, HA1 3UJ, U.K.

Materials and Methods

Materials were obtained as described earlier [4,5] and egg phosphatidylcholine (type VII-E),

cholesterol, fatty acids, ferrozine (3-(3-pyridyl)-5,6-bis(4-phenylsulphonic acid)-1,2,4-triazine) and Ca^{2+} ionophore A23187 (free acid) were obtained from Sigma. [1-^{14}C]Oleic acid and [4-^{14}C]cholesterol were obtained from Amersham International (Amersham, U.K.). $^{59}Fe^{2+}$ incubation media were prepared essentially as described previously [3]. Ca^{2+} ionophore (A23187) was dissolved in dimethylsulphoxide/ethanol (1 : 1, v/v).

Solvent extraction was performed essentially as described in Ref. 1. 500 μl iron-labelled medium (100 μM $^{59}Fe^{2+}$/2 mM sodium ascorbate/0.1 M NaCl/0.1 M mannitol/20 mM Na-Hepes buffer (pH 7.25)) was incubated with various concentrations of oleic acid (added as a 50 mM solution in ethanol) for 10 min at 37 °C. Solvent (200 μl of either chloroform/methanol (2 : 1, v/v) or toluene/butanol (3 : 1, v/v) was added and the mixture vortexed, centrifuged (10000 × *g*, 10 min) and 100 μl of the organic phase removed and counted for ^{59}Fe.

Liposomes were prepared by hydrating lipid films with 0.5 ml of 10 mM ferrozine/10 mM sodium ascorbate/0.1 M NaCl/0.1 M mannitol/20 mM Na-Hepes buffer (pH 7.2), by agitation with glass beads. The mixtures were sonicated on ice (5 × 10 s bursts separated by 10 s and 1 min after the first three bursts: 100 W, MSE sonic disintegrator) then applied to a Sephadex G-50 column (1 × 30 cm) eluted with 0.1 M NaCl/0.1 M mannitol/20 mM Na-Hepes (pH 7.2) at 6 °C. Fig. 1 shows elution profiles for cholesterol, oleic acid, ferrozine and liposomes (as detected by light scattering) from such a column. The included ferrozine peak, [^{14}C]cholesterol, [^{14}C]oleic acid and main light-scattering peak all coincide at the void

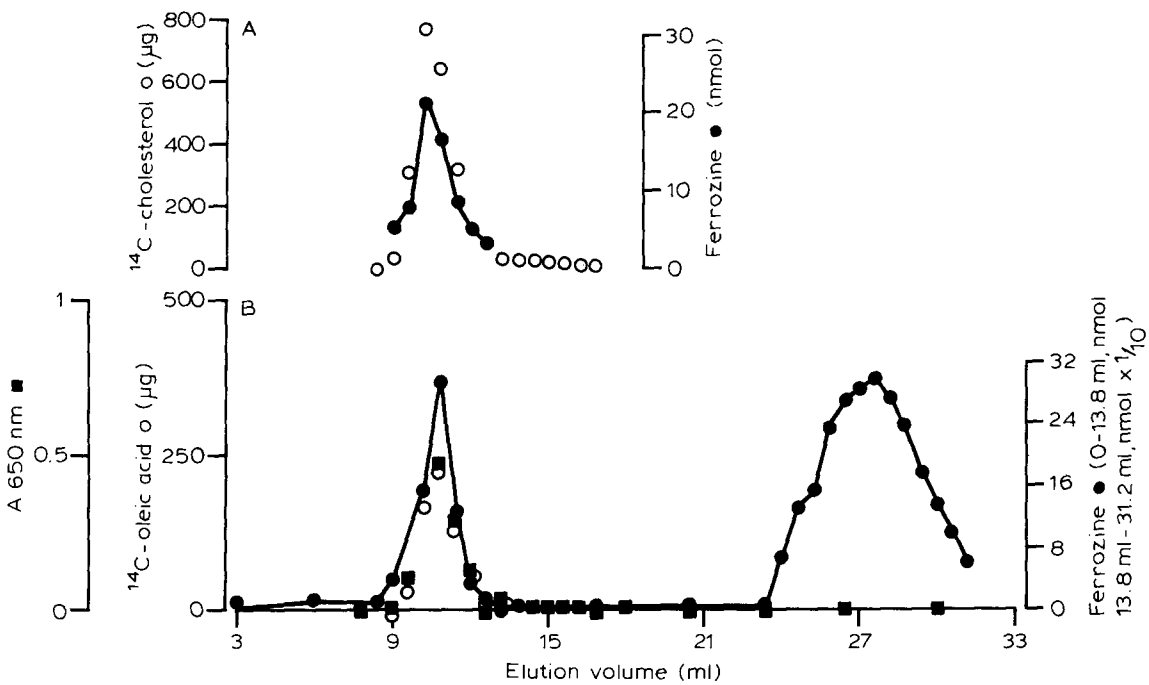

Fig. 1. Elution profiles for ferrozine, [^{14}C]oleic acid, [^{14}C]cholesterol and liposomes (light scatter at 650 nm) from Sephadex G-50 column used for liposome preparation. 1 mg of oleic acid (with (B) or without (A) 10^5 cpm of [1-^{14}C]oleic acid), 10 mg of egg phosphatidylcholine, 2.5 mg of cholesterol (with (A) or without (B) 10^5 cpm of [4-^{14}C]cholesterol) were hydrated in 0.5 ml of 0.1 M NaCl/0.1 M mannitol/20 mM Na-Hepes (pH 7.25)/10 mM ferrozine/10 mM sodium ascorbate and sonicated (see Methods). An aliquot was retained for counting and the remainder was applied to a 1 × 30 cm column of Sephadex G-50 equilibrated and eluted with the above buffer, lacking ferrozine and ascorbate, at 0.6 ml/min (6 °C). Fractions were collected (0.6 ml) and the absorbance at 650 nm and ^{14}C content determined. Ferrozine was determined by adding 900 μl of 100 μM $FeCl_3$, 100 μM HCl, 200 μM sodium ascorbate, 0.1% Triton X-100 to 100 μl of column fraction, incubating for 1 h at 37 °C then determining the $A_{562\,nm}$. Recoveries of oleic acid and cholesterol were 93 and 100%, respectively.

volume of the column suggesting that the lipid composition of the final liposomes reflects the original mixture. The fatty acid composition of liposomes was therefore calculated from the composition of the original mixture.

The peak liposome-containing fractions were pooled and Fe uptake followed at 37 °C by recording the increase in absorbance at 562 nm (of the Fe^{2+}-ferrozine complex) with a Perkin-Elmer 557 dual beam spectrophotometer. Liposomes (400 μl portions) were preincubated at 37 °C for 10 min. At $t = 0$, 400 μl of a freshly prepared solution of 200 μM Fe^{2+}/4 mM sodium ascorbate/0.1 M NaCl/0.1 M mannitol/20 mM Hepes (final pH 7.2) (preincubated for 5 min at 37 °C) was added and absorbance at 562 nm measured at 37 °C. Ca^{2+} ionophore A23187 (5 μM) was added to mediate Fe^{2+} uptake by control liposomes [1] so as to demonstrate the uptake endpoint and confirm the integrity of the liposomes. Uptake endpoints for both fatty acid-containing and control liposome preparations corresponded to approx. 9 μM total uptake of Fe^{2+}. Glass cuvettes were washed with water and ethanol before use. Because of the exceptional sensitivity of the transport rate to pH, medium pH was routinely measured after rate determinations. Where necessary medium pH was carefully adjusted to balance the effects of varying e.g. Fe/ascorbate concentrations on pH. Results shown are generally representative experiments that could be reproduced on three separate occasions.

Mathematical modelling of transport kinetics was based on a steady-state assumption analogous to that of Briggs and Haldane for enzyme kinetics [9]. A general equation for transport of one Fe by N ligand molecules (L) was derived from the model

$$\mathrm{Fe(out)} + N\,\mathrm{L} \underset{k_2}{\overset{k_1}{\rightleftarrows}} \mathrm{FeL}_N \xrightarrow{k_3} \mathrm{Fe(in)} + N\,\mathrm{L}$$

yielding

$$([\mathrm{Fe}]([\mathrm{L}] - N \cdot V)^{(N/K)}) - V(1 + ([\mathrm{L}] - N \cdot V)^{(N/K)}) = 0$$

where K is $(k_2 + k_3)/k_1$, V is k_3 times the maximum possible concentration of FeL_N and [Fe] and [L] are the total concentrations of Fe and L, respectively. This equation was solved by Newtons' method using BASIC programs, modified where necessary to include the effects of pH (assumed to affect total Fe and L concentrations) or inhibitor competitive with Fe for L. Protons were not assumed to compete with Fe for L as protonated fatty acids are lipid soluble and may be sequestered away from the Fe.

Results and Discussion

A first requirement for a membrane Fe carrier would be the capacity to render Fe lipid soluble. Fig. 2 shows that oleic acid can form a lipid soluble complex with Fe as shown by extraction into toluene/butanol (3:1, v/v) or chloroform/methanol (2:1, v/v). Other fatty acids (e.g. palmitic) were also found to show this property.

Fig. 3 shows that the liposomes prepared from phosphatidylcholine/cholesterol alone are impermeable to Fe^{2+} but are competent to express transport when ionophore A23187 is added. It can be seen that incorporation of small quantities of oleic acid facilitates transport of Fe^{2+} across a phosphatidylcholine/cholesterol liposome membrane. Experiments in which liposomes were preincubated for up to 1 h reveal that the permeability increase is selective for the metal ion in that no leakage of intraliposomal ferrozine (M_r 471) occurred. This suggests that non specific detergent effects of the fatty acid are not important at these

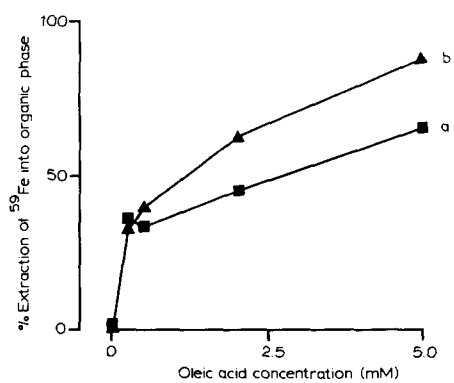

Fig. 2. Extraction of Fe-oleate complexes by organic solvents. Solvent extraction was performed as described in Methods using 100 μM ^{59}Fe, 2 mM sodium ascorbate, 0.1 M NaCl, 0.1 M mannitol, 20 mM Na-Hepes (pH 7.25) and (a) chloroform/methanol (2:1, v/v) or (b) toluene/butanol (3:1, v/v).

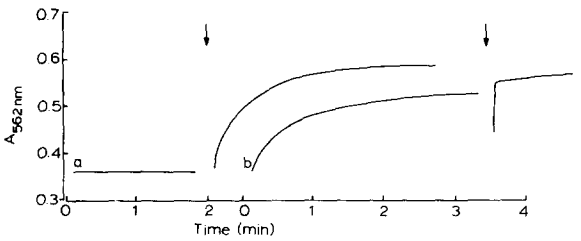

Fig. 3. Effect of oleic acid on Fe^{2+} transport by phosphatidylcholine/cholesterol liposomes. Liposomes were prepared as described in Methods without (curve a) or with (curve b) oleic acid (8% of total lipid). Liposomes were mixed with Fe/ascorbate medium and A_{562nm} recorded as described in Methods. Arrows indicate the points at which Ca^{2+} ionophore A23187 (5 μM) was added.

concentrations. The absence of transport in the absence of ionophore and fatty acid demonstrates that ferrozine itself is not lipid soluble enough to act as a membrane carrier for Fe^{2+}.

The time-courses for Fe^{2+} uptake resemble exponentials. However, log plots revealed non-linearities, therefore further kinetic analysis was performed with initial rates, as linear portions could usually be obtained over the period 5–20 s after starting incubations. Fig. 4 shows the effect of varying the duration of the sonication time in the preparative procedure for oleic acid-containing liposomes on the initial Fe^{2+} transport rate and included volume. After a sharp rise the initial rate of uptake is relatively independent of the sonication time. The initial rise is presumably associated with conversion of multilamellar to unilamellar liposomes. Electron microscopy of the liposome preparations used in the uptake experiments revealed a heterogeneous collection of small spherical bodies (50–100 nm diameter) in both fatty acid-free and fatty acid-containing samples. This size is consistent with liposomes of the unilamellar type.

Transport of Fe^{2+} by various levels of different fatty acids

Fig. 5 shows the dependence of initial Fe^{2+} uptake rates on fatty acid content of liposomes for several naturally occurring fatty acids. All fatty acids investigated showed a sharp increase in rate when incorporation exceeded approx. 4% of total lipid. This behaviour could be fitted by assuming a high order dependence of transport rate on fatty acid concentration, that is, that more than one fatty acid molecule is necessary to effect the transport of a single Fe ion. The data could be well fitted with 3rd or 4th order models (that is 3 or 4 fatty acids per Fe) by assuming steady-state formation and breakdown of (fatty acid)-Fe complexes and initial rate conditions (N.B. fatty acid concentration was not assumed to be small compared with Fe concentration). The stoichiometry required for this model may suggest that Fe is

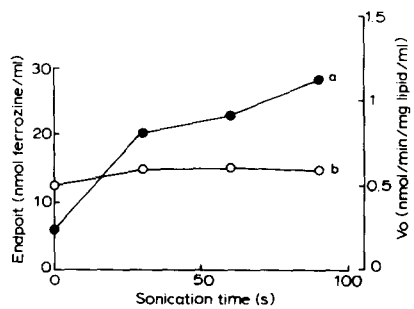

Fig. 4. Effect of sonication time on initial rate of Fe^{2+} transport and uptake endpoint. Lipid mixtures as in Fig. 1 only lacking ^{14}C-labelled lipids and with 0.75 mg oleic acid were prepared as in Fig. 3 and the sonication time was varied as indicated. Each group of three bursts was separated by a cooling period of 1 min. Initial transport rates (V_0) (a) and endpoints (b) were determined in duplicate with incubation medium as in Fig. 3 at 37 °C.

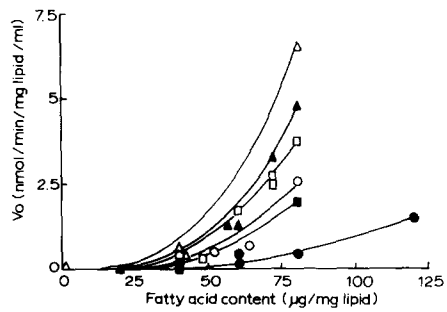

Fig. 5. Effect of varying fatty acid levels on liposome Fe^{2+} transport. Liposomes were prepared as in Fig. 3 only with differing levels of fatty acids (palmitic (●), myristic (○), arachidonic (■), linoleic (△), linolenic (□) and oleic (▲)). Data points are means of duplicate determinations of initial transport rate. The lines are obtained from a steady-state model (see Methods) assuming three fatty acids involved per Fe transported. Incubation conditions were as in Fig. 3.

transported as Fe^{3+}, presumably in rapid equilibrium with Fe^{2+}. However, preparation of liposomes lacking ascorbate inside did not abolish transport (ferrozine does not form a purple complex with Fe^{3+}). Another possible explanation for this dependence on fatty acid level is that effects of fatty acids on membrane fluidity [10] occur especially at the higher levels.

The curves shown in Fig. 5 may be used to order the fatty acids on the basis of Fe^{2+} transport giving linoleic > oleic > linolenic > myristic > arachidonic > palmitic. In experiments where mixtures of fatty acids were employed (40 μg palmitic/mg lipid plus various oleic acid levels or various levels of an equimolar mixture of palmitic, stearic, myristic, arachidonic, linoleic, linolenic, arachidonic and oleic acids) no synergism was observed, that is the rates observed were the result of simple addition of the rates noted in Fig. 5.

Factors affecting free Fe^{2+} in Fe^{2+}/ascorbate mixtures near pH 7.0

It became apparent from studies in which pH or Fe/ascorbate concentration were varied that the Fe^{2+} transport rate was partially determined by free Fe^{2+} concentration (see below). In order to fully interpret the kinetics of transport, an understanding (even empirical) of factors affecting free Fe^{2+} in Fe^{2+}-ascorbate mixtures was necessary. We found that the published association constants for Fe^{2+}/ascorbate/H^+ interactions [11–13] were adequate to explain the variation of the Fe^{2+}-ascorbate complex concentrations (observed by absorbance of the violet complex at 533 nm [11,13]) at various ascorbate: Fe ratios, Fe-ascorbate concentrations and pH (not shown). These values, however, predict that the majority of Fe in the solutions employed here should be free Fe^{2+} and that only small changes should occur over the pH range 6.5–7.5. This could not explain the very sharp decline in Fe^{2+} transport observed above pH 7.0 (see below). We therefore used ferrozine to probe the free Fe^{2+} concentration in our media. Fig. 6A shows the time course for appearance of Fe^{2+}-ferrozine complex ($A_{562\,nm}$) at 37 °C in a medium similar to that employed for Fe^{2+} transport studies. A small portion of the Fe reacts within 5 s, the remainder reacts slowly, taking more than 1 h at pH 7.25. We interpret this

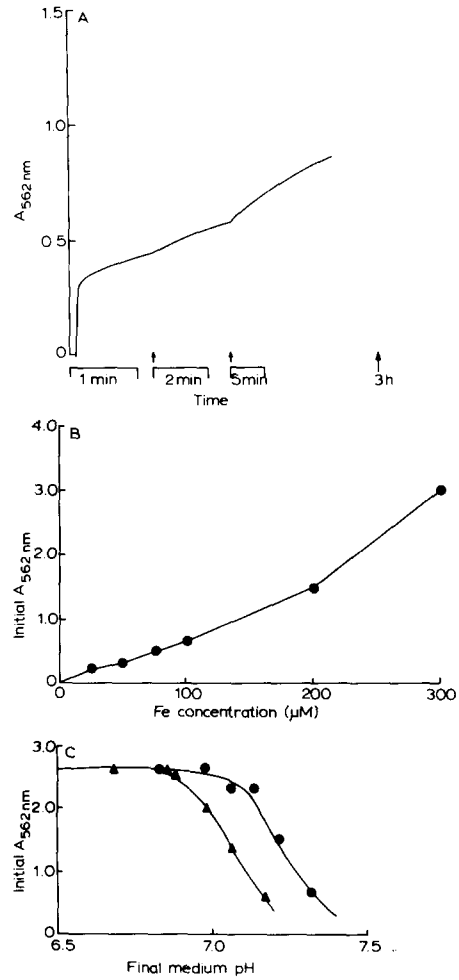

Fig. 6. Determination of free Fe^{2+} using ferrozine: effect of medium pH and Fe/ascorbate level. Fe/ascorbate incubation media were prepared and preincubated as described in Fig. 3. At $t = 0$ an equal volume of 2 mM ferrozine in 0.1 M NaCl, 0.1 M mannitol, 20 mM Hepes (pH 7.25) (preincubated at incubation temperature for 5 min) was added and recording to $A_{562\,nm}$ commenced at $t = 5$ s. (A) Final medium Fe was 100 μM, ascorbate 2 mM, pH 7.25 and incubation temperature was 37 °C. The alterations in the time constant are indicated by the arrows. (B) The extrapolated $t = 0$ absorbance from time-courses such as in (A) were determined at various final medium Fe concentrations (Fe: ascorbate ratio 1 : 20, temperature 37 °C, final pH 7.15). (C) The initial absorbance was determined at various final medium pH at 37 °C (▲) or 22 °C (●). The final pH of incubation mixtures was determined at the incubation temperature. Medium pH was varied by altering the pH of the Fe/ascorbate-containing mixtures through the use of Hepes buffers. In (B) and (C), the higher absorbances were measured with a 1 mm path-length cell but all data are expressed for a path-length of 10 mm. The lines in B are of the form $A_{562\,nm} = 2.56/(pK_{app} \cdot 6 \cdot 10^{6 \cdot (pH-14)} + 1)$ with pK_{app} values of 6.945 and 6.77 at 37 and 22 °C, respectively.

time-course to represent Fe^{2+} reacting immediately and Fe^{2+}-ascorbate and/or other Fe complexes present reacting more slowly. The nature of these other Fe complexes is not known but they could conceivably be $Fe(OH)_2$, Fe^{3+}-ascorbate [11], Fe^{3+} hydroxide species or Fe-Hepes complexes. Studies in which Hepes concentration was varied 10-fold at constant pH without effect on apparent free Fe^{2+} excluded the latter possibility. Experiments in which the ferrozine concentration was varied over a 20-fold range similarly revealed that the complexing agent did not limit the apparent free Fe^{2+} concentration.

We found that the dependence of the apparent free Fe^{2+} concentration on pH (above pH 6.9) and total Fe/ascorbate level resembled the dependencies of Fe^{2+} transport observed with liposomes, including the temperature shift in the pH dependence (see below), when the experiments were performed in exactly the same way as those with liposomes (substituting ferrozine for liposomes).

The dependence of free Fe^{2+} on final assay pH could be empirically fitted by a 6th order dependence on OH^- (Fig. 6). The dependence arises from an actual approximate 3rd order dependence (β_3 (app) 10^{21} at 37 °C) on the pH of the preincubation medium. This is distorted to an apparent 6th order dependence in final medium pH due to the buffering capacity of the ferrozine-containing medium.

Another property of apparent free Fe^{2+} observed in these experiments was a decrease with increasing age of the Fe/ascorbate solutions. A similar decline in Fe^{2+} transport in experiments with brush-border membrane vesicles and liposomes had also been observed. A standard preincubation time for Fe/ascorbate solutions was employed in these and previous studies [3,4].

Further studies of the nature of the Fe-species present in Fe/ascorbate solutions near neutral pH is underway, although for the study of fatty acid-mediated transport of Fe^{2+} the empirical observations noted above are sufficient.

Dependence of Fe^{2+} transport on medium pH and Fe^{2+} concentrations

Fig. 7 shows the dependence of oleic acid-mediated Fe^{2+} transport on total medium Fe concentration at constant ascorbate/Fe ratio. Comparison of this with Fig. 6B suggests that free Fe^{2+} is the primary determinant of the transport rate in these circumstances. The model used to fit the dependence on fatty acid level (Fig. 5, Methods) predicts a linear dependence of rate on Fe^{2+} concentrations in this range and at this fatty acid level for all 'K_m' values greater than 10^{-7} M[3].

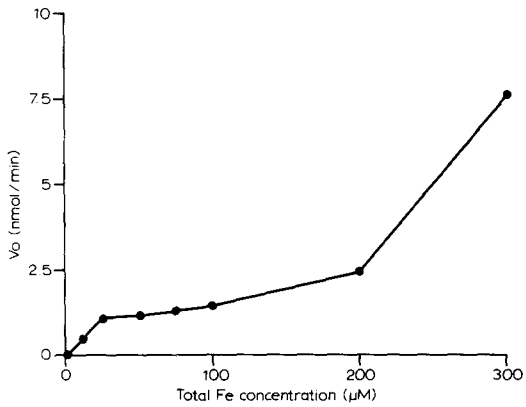

Fig. 7. Dependence of oleic acid catalysed Fe^{2+} transport on medium Fe/ascorbate concentration. Liposomes were prepared as in Fig. 3 except that 1.5 mg oleic acid, 24 mg egg phosphatidylcholine and 5 mg of cholesterol were hydrated with 1 ml of 0.1 M NaCl, 0.1 M mannitol, 20 mM Hepes (pH 7.25), 10 mM ferrozine, 10 mM sodium ascorbate and sonicated for 9×10 s bursts. Other preparative steps and transport determination were as in Fig. 3 (incubation temperature 37 °C) except the Fe concentration was varied as shown at constant ascorbate: Fe ratio of 20:1.

Fig. 8 shows the pH dependence of the initial rate of Fe^{2+} transport by palmitic acid. Similar sharp optima at pH 6.9–7.0 were observed with oleic acid, linoleic acid and with an equimolar mixture of 7 fatty acids (see above).

In order to fit the pH dependence data, a model involving a 6th order dependence on fatty acid was required (Fig. 8). The reason for this apparent involvement of more fatty acids than was inferred from the dependence on fatty acid level (Fig. 5) is not clear. The possibility that pH gradients are important seems unlikely, as the fatty acids themselves would tend to collapse any such gradients. Also, experiments in which oleic acid-mediated transport at pH 6.7 (37 °C) was studied in the presence and absence of 100 μM 2,4-dinitrophenol revealed no effect.

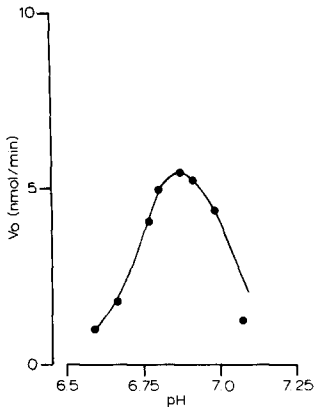

Fig. 8. Dependence of Fe transport on medium pH. Liposomes were prepared as in Fig. 7 with 2.2 mg palmitic acid instead of oleic acid. Transport was determined as in Fig. 3 at 37°C. Medium pH was varied as in Fig. 6C. Final medium pH was determined at the incubation temperature after the transport rate determination. The line was obtained from the model described in Methods modified using the lines derived as in Fig. 6C. Six fatty acids with pK_{app} values of 6.93 per Fe were assumed in order to fit the ascending slope at low pH.

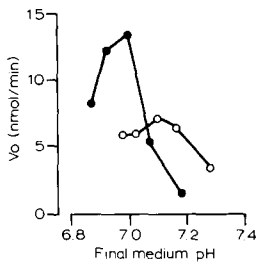

Fig. 9. Effect of reducing incubation temperature on Fe^{2+} transport by oleic acid. Liposomes were prepared as in Fig. 7 and medium pH varied as in Fig. 8. The final pH of each mixture was determined at the incubation temperature after the transport determination (○, 22°C; ●, 37°C).

The ascending slope of the optimum at acid pH may be attributed to the fatty acid ionization. We have measured apparent Fe binding by oleic acid as a function of pH and noted a steep rise over the pH range 6.5–7.2 [14]. It is noteworthy that apparent Fe binding is much less sensitive to Fe concentration than Fe transport (see Ref. 14 and compare Fig. 7), leading to an upward shift in the pH optimum for binding. This difference may arise because the apparent Fe^{2+} binding measured in Ref. 14 probably includes the solubility product for Fe^{2+}-fatty acid soaps. The pK values of all fatty acids are believed to be similar (4.9 [15]) although the actual value is difficult to measure due to their insolubility: our data suggest pK values close to 7.0 (see Fig. 8). The pK values may also be affected by their environment in, for example, a membrane. It is noteworthy that fatty acid pK values of 7.25–7.4 have been reported in phosphatidylcholine liposomes [16].

Fig. 9 shows the dependence of oleic acid-mediated transport of Fe^{2+} on medium pH at two temperatures. The maximal rate of transport decreases with decreasing temperature, the Q_{10} being approx. 1.3. It is noteworthy that the increase in free Fe^{2+} with reduced temperature above pH 7.0 (see Fig. 6C) results in an overall increase in transport rate with decreasing temperature at the high pH values.

Inhibition of oleic acid-mediated Fe^{2+} transport

NaCl was found to be a weak inhibitor of Fe^{2+} transport by brush-border membrane vesicles [3,4,17]. It might be expected that Na^+ would inhibit fatty acid-mediated transport since Na^+ is known to form complexes with fatty acids. Experiments employing high NaCl concentrations were complicated by light scattering changes brought about by liposome shrinkage. It was clear, from the absence of purple Fe^{2+}-ferrozine complex, that transport was markedly inhibited by NaCl. Preincubation of liposomes with NaCl removed osmotic effects allowing unhindered measurement of absorbance changes. Fig. 10 shows that NaCl is a weak inhibitor of oleic acid-mediated Fe^{2+} transport, the apparent K_i being 10^4-fold higher than the Fe concentration employed.

$CoCl_2$ was found to have no effect on initial rates of oleic acid-mediated Fe^{2+} transport (Fig. 11b) at concentrations (1 mM) which have been reported to be inhibitory to brush border membrane vesicle Fe^{2+} transport [3]. Preincubation of liposomes with 1 mM $CoCl_2$ led to complete inhibition of transport (Fig. 11c). When Fe^{2+}, Co^{2+} and ferrozine were mixed in the absence of liposomes, it was found that no Fe^{2+}-complex formation was observed if Co^{2+} was added to the ferrozine first, whereas when Fe^{2+} was added first, normal complex formation occurred. These observations suggest that Co^{2+} does not greatly af-

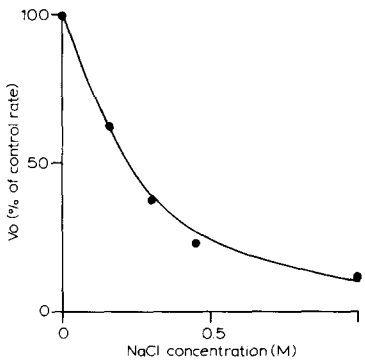

Fig. 10. Inhibition of oleic acid-mediated Fe transport by NaCl. Liposomes were prepared as in Fig. 7 and preincubated for 10 min in buffer containing the indicated NaCl concentrations. An equal volume of 200 μM Fe, 4 mM sodium ascorbate, 0.1 M mannitol, and 20 mM Hepes (pH 7.25), containing the indicated NaCl concentration (preincubated 5 min at 37 °C) was added to commence the transport determination. The line was obtained using the model as in Fig. 5 assuming a K_i of 0.9 M.

fect Fe^{2+} transport by fatty acids but Co^{2+} entry into the liposomes inhibits subsequent Fe^{2+} accumulation by formation of a kinetically stable Co^{2+}-ferrozine complex. These findings emphasize that, depending on circumstances, both initial transport and subsequent binding steps can determine the observed Fe^{2+} transport rate.

In isolated brush-border membrane the fatty acid level [5–7] falls within the range found to catalyse rapid Fe^{2+} transport in liposomes. The inhibition of transport by NaCl and the pH optimum both resemble the effects seen in brush-border membrane vesicles [3,4,17,18]. Fe^{2+} transport by brush-border membrane vesicles is partially inhibited by Co^{2+}, unlike fatty acid-mediated transport. It should be noted, however, that the studies with vesicles did not determine whether the effect of Co^{2+} was an initial rate effect or an effect on the subsequent intravesicular binding of Fe. In this work, the liposome phospholipid/cholesterol ratios were selected to resemble those of brush-border membrane vesicles [5,6]. However, the effect of the other phospholipids and glycolipids, present in the brush-border membrane, on Fe^{2+} transport remains to be investigated. These observations may provide a molecular basis for Fe^{2+} transport across specific membranes, although the pH optima suggests this mechanism is only important at neutral pH values. It is also possible that permeability of membranes to other cations can be affected by free fatty acids.

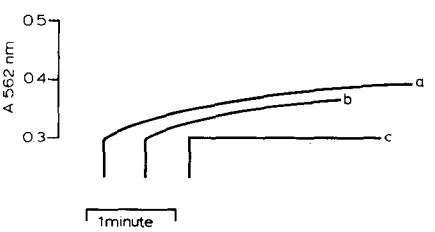

Fig. 11. Effect of Co^{2+} on oleic acid-mediated Fe^{2+} transport. Time-courses for Fe^{2+} transport in the absence (a) and presence (b) of 1 mM added $CoCl_2$. Other conditions were as in Fig. 3 (incubation temperature 37 °C). In (c) liposomes were preincubated for 10 min at 37 °C with 1 mM $CoCl_2$ before addition of Fe/ascorbate mixture (containing 1 mM $CoCl_2$).

Conclusions

Non-esterified fatty acids can catalyse rapid transport of Fe^{2+} across artificial lipid bilayers at levels observed in certain biological membranes. Kinetic modelling suggests the involvement of a complex containing more than one fatty acid per Fe. The effects of pH and NaCl on transport resemble effects seen with isolated brush-border membrane Fe^{2+} transport.

Acknowledgements

We thank Miss P. McAree and Mrs. S. Kingsley for typing this manuscript and H. Grindley for electron micrographs.

References

1 Young, S.P. and Gomperts, B.D. (1977) Biochim. Biophys. Acta 469, 281–291
2 Llewin, R. (1984) Science 225, 401–402
3 Simpson, R.J. and Peters, T.J. (1985) Biochim. Biophys. Acta 814, 381–388
4 Simpson, R.J. and Peters, T.J. (1986) Biochim. Biophys. Acta 859, 227–236
5 Hauser, H., Howell, K., Dawson, R.M.G. and Bowyer, D.E. (1980) Biochim. Biophys. Acta 602, 567–577
6 Christiansen, K. and Carlsen, J. (1981) Biochim. Biophys. Acta 647, 188–195

7 Forstner, G.G., Tanaka, K. and Isselbacher, K.J. (1968) Biochem. J. 109, 51–59
8 Nelson, G.J. (1967) J. Lip. Res. 8, 374–379
9 Briggs, G.E. and Haldane, J.B.S. (1925) Biochem. J. 19, 338–339
10 Pauls, J.P., Mackay, A.L. and Bloom, M. (1983) Biochemistry 22, 6101–6109
11 Ulmgren, P. and Wahlberg, D. (1974) Acta. Chem. Scand. 28A, 631–637
12 Sillen, L.G. and Martell, A.E. (1971) Special Publication No. 25, The Chemical Society, Burlington House, London
13 Plug, C.M., Dekker, D. and Bult, A. (1984) Pharmaceutisch Weekblad 6, 245–248
14 Simpson, R.J. and Peters, T.J. (1987) Biochim. Biophys. Acta 898, 181–186
15 Garvin, J.E. and Karnovsky, M.L. (1956) J. Biol. Chem. 221, 211–222
16 Ptak, M., Egret-Charlier, M., Sanson, A. and Bouloussa, O. (1980) Biochim. Biophys. Acta 600, 387–397
17 Simpson, R.J. and Peters, T.J. (1986) Biochim. Biophys. Acta 856, 109–114
18 Cox, T.M. and O'Donnell, M.W. (1981) Biochem. J. 194, 753–759