Studies on the Major Intermediate Transit Iron Complex in Human Placenta

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Abstract

Ultrafiltrable iron ion in human term placenta was eluted in association with lactate on a Sephadex G-25 column. A major ligand in the intermediate iron pool was deduced to be lactate, as demonstrated by the UV absorption spectrum and ^{13}C NMR spectrum. The paramagnetic shift of the methine proton of the lactate–iron complex, as demonstrated by the ^{1}H NMR spectrum, disappeared after removal of iron by Chelex 100 treatment. Paper electrophoresis at pH 4.2 revealed that, beside the major iron–lactate complex, a minor one migrated to the anode as fast as did the iron–citrate complex. The present data suggested that, together with a large excess of lactate, iron ion might flux to the fetal circulation across the placenta.

Introduction

There is a rapidly increasing amount of iron transported unidirectionally from mother to fetus across the placenta as fetal growth increases. Iron in the maternal circulation is taken up by the placental cells and rapidly transported to the fetal circulation so that no accumulation of ^{59}Fe-containing compounds can be measured in the placenta [1–3]. ^{59}Fe is associated with either one of three different forms: transferrin, ferritin and a third form characterized by its small molecular weight. The involvement of this last form suggests the presence of an intracellular chelator operating to shuttle iron, although its exact nature is not known [4].

A reducing agent such as ascorbate appears to fulfill a role in iron transfer effectively, although it is enhanced greatly if any chelator is also present. Citrate, sugars, amino acids and nucleotides may also function as intermediate ligands in iron transfer [5]. As mentioned by Jacobs [6, 7], there is a striking contrast between the detailed knowledge of iron-carrying proteins and the ignorance regarding the intermediate iron pool. There is, however, no reason why the iron pool consists of a single type of complex. All the component compounds will share some properties.

This paper deals with a major iron complex in the ultrafiltrate of human term placenta.

Experimental

Fresh human term placentas were used within one hour after normal delivery. After removal of the chorionic and amniotic membranes, they were washed with saline solution to remove as much blood as possible. Placental tissue was homogenized with two volumes of redistilled iron-free water or phosphate buffer (0.05 M, pH 7.2) with or without butanol treatment [8]. After centrifugation, the supernatant was ultrafiltrated through an Amicon Diaflor Membrane YM5 which permitted the filtration of substances with molecular weights lower than 5000.

The iron content was determined by an atomic absorption spectrophotometer, Nippon Jarrell Ash AA-855, or by the 2,2′-dipyridyl method [9], ferrous directly and ferric after reduction with ascorbic acid. Lactate was measured according to the Olsen's enzymatic method [10] with a JASCO EP-550 Spectrofluorometer. The ^{13}C NMR and ^{1}H NMR spectra were recorded at 50.1 MHz and 199.5 MHz, respectively, with a JEOL FX-200 FT-NMR Spectrometer.

The ultrafiltrate (160 ml from 100 g tissue) was lyophilized and then suspended in 80% methanol to remove the added phosphate and insoluble organic materials. The methanol layer was evaporated to dryness and dissolved in 6 ml of redistilled water. Each 2 ml solution was applied on a Sephadex G-25 column (2.6 × 80 cm) with or without an addition of $FeCl_3$ or $FeSO_4$ at the final concentration of 0.5 mM. It was eluted with redistilled iron-free water at flow rate of 50 ml per hour and 3 ml fractions were collected. The iron-containing fraction was passed through a CM-cellulose column (2.6 × 26 cm) which was prepared by washing with 0.2 M NaOH and 0.2 M HCl and finally with redistilled water. The pass-through fraction was used for the chemical and physicochemical analyses and also, after concen-

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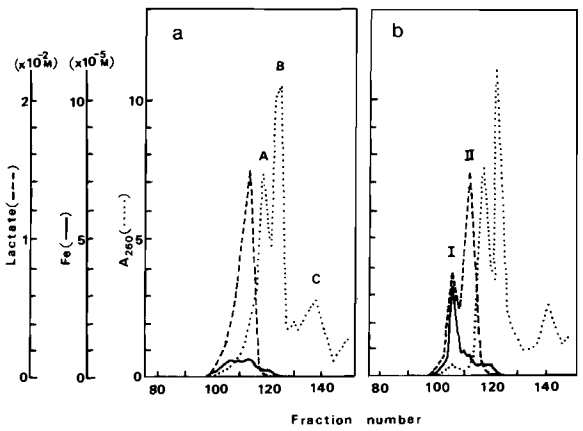

Fig. 1. Sephadex G-25 gel filtration of placental ultrafiltrate: (a) without Fe; (b) 5×10^{-4} M $FeCl_3$ added.

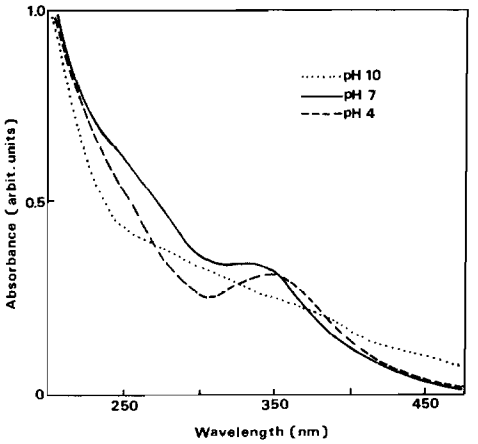

Fig. 2. pH change in absorption spectrum of peak I.

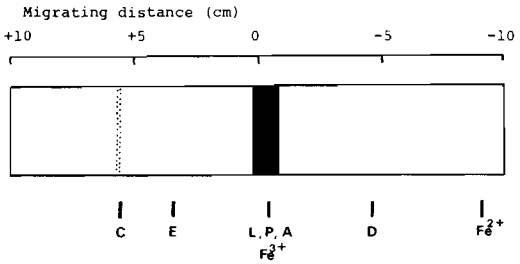

Fig. 3. Paper electrophoresis at pH 4.2. After electrophoresis, the paper strip was dried and sprayed with 2% dipyridyl in ethanol and then with or without 5% ascorbic acid in methanol. The migrating distances of known iron complexes and free iron ions are indicated below as references: C, Fe^{3+}–citrate; E, Fe^{3+}–EDTA; L, lactate; P, pyruvate; A, acidic amino acid; D, Fe^{2+}–dipyridyl. The migration of iron complexes with L, P and A does not differ on the valency state.

tration, for the paper electrophoresis which was run in 0.05 M acetate buffer, pH 4.2, at 400 V per 30 cm for 1 h in the horizontal-type apparatus.

Bio-Rad Chelex 100 (200–300 mesh, sodium form) was used to remove iron when necessary. All reagents used were of analytical grade. Glasswares used for the preparation of reagents and the performance of analyses were soaked in 6 M nitric acid overnight and rinsed thoroughly with redistilled iron-free water.

Results

The amount of iron which was extractable in three kinds of ultrafiltrates (redistilled water and phosphate buffer with or without butanol treatment) was 50 μg, 53 μg and 70 μg per 1 kg of placental tissue, respectively. These values were not on different scales although the small molecular weight iron complexes in rabbit placenta at 28–29 days pregnancy were suggested [8] to be integral parts of cell membranes because they were water-soluble only after butanol treatment.

Figure 1 shows the elution profiles of placental ultrafiltrate extracted with phosphate buffer after butanol treatment, with or without an addition of iron ion, on a Sephadex G-25 column. Iron ion is eluted as a complex at the elution position of lactate just before the ascorbate-containing peak A. Peaks B and C contain uracil and hypoxanthine, respectively. Three kinds of ultrafiltrates gave nearly the same elution profile. For the ferric ion-added ultrafiltrate, the iron complex mainly appears in the peak I of which elution position is much faster than that of lactate peak II. When 2 ml mixture of 5 mM ferric chloride and 500 mM sodium lactate was run on the same gel filtration system, 80% of applied iron was recovered at the same elution position as that of peak I. With an addition of 50 mM or less sodium lactate, however, most of applied iron was retained tightly in the column.

The absorption spectrum of peak I fraction and its pH change (Fig. 2) are just the same as those of the authentic Fe^{3+}–lactate mixture, indicating an absorption maximum at 325 nm (pH 7) and at 345 nm (pH 4). The peak II fraction did not show such a characteristic absorption spectrum. As the placental ultrafiltrate contains an appreciable amount of reducing substances such as ascorbate, added ferric ion was reduced mostly to the ferrous state, although the added ferrous ion received a partial oxidation during the chromatographic procedure. Thus, the elution profile is not significantly different between two valency states of added iron ion.

The paper electrophoresis pattern at pH 4.2 (Fig. 3) indicates that the major iron complex of placental ultrafiltrate remains near the applied position and a minor one migrates to the anode as fast as Fe^{3+}–citrate complex. The migrating distance of the major component is the same as those of

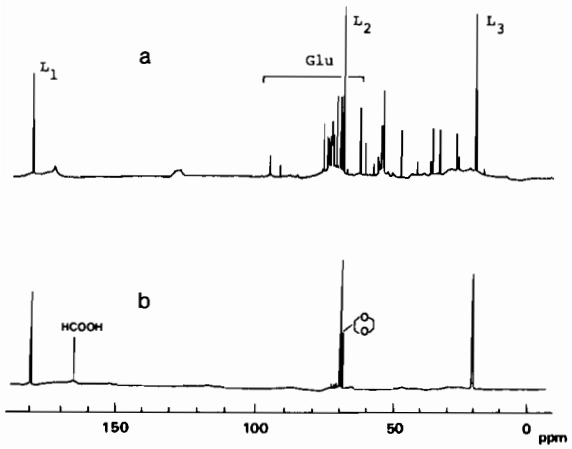

Fig. 4. ^{13}C NMR spectra: (a) placental ultrafiltrate; (b) iron–lactate fraction on a CM-cellulose column. Glu: glucose; L_1, L_2, L_3: lactate. Dioxane and formic acid are used as external references.

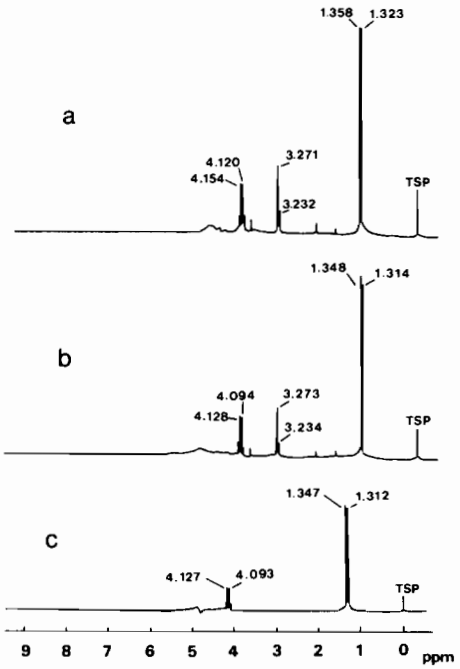

Fig. 5. ^1H NMR spectra of G-25 fractions: (a) peak I; (b) peak I after Chelex 100 treatment; (c) sodium lactate. TSP: 3-(trimethylsilyl)propionate$_{d-4}$.

iron complexes with lactate, α-ketoacid or acidic amino acid.

The ^{13}C NMR spectrum of placental ultrafiltrate (Fig. 4a) reveals the lactate (14 mmol per 1 kg placental tissue), glucose and unknown signals as the major organic compounds. In the peak I pass-through fraction on a CM-cellulose column, almost all of lactate together with iron ion was recovered of which ^{13}C NMR signals (Fig. 4b) are exclusively composed of those of lactate.

Figures 5a, b and c indicate the ^1H NMR spectra of the peak I pass-through fraction before and after Chelex 100 treatment and sodium lactate as reference, respectively. In the spectrum of peak I (Fig. 5a), the doublet (1.32–1.36 ppm) and the quartet (4.08–4.19 ppm) resonances are assigned to methyl and methine protons of lactate (Fig. 5c). The assignment of resonances at 3.271, 3.232 and the others are uncertain. The paramagnetic shift of methine proton of lactate (+0.027 ppm) is much greater than that of methyl proton (+0.011 ppm). This is consistent with the information that the α-hydroxyl group together with carboxyl group takes part in the binding sites to metal ions [11, 12]. After Chelex 100 treatment, the signal of methine proton becomes similar to that of free lactate (Fig. 5b). The other signals do not receive any paramagnetic effect. These results indicate the preferential interaction of iron ion with lactate in the placental ultrafiltrate.

Discussion

Lactate is one of the major organic compounds in the placental ultrafiltrate as demonstrated on the ^{13}C NMR spectrum. It forms a multiligand iron complex as shown by the absorption spectrum and by the paramagnetic shift in the ^1H NMR spectrum. The placenta provides lactate to the fetal circulation in sufficient quantities to account for a quarter of fetal oxidative metabolism [13]. The lactate concentration in cord blood is significantly greater than that in maternal blood [14, 15]. And there is a big difference between the venous and arterial concentration in the cord vessels. Thus, a large amount of lactate may flux together with iron ions to the fetal circulation.

In gel chromatography, the mixture of iron ion and lactate may undergo a stripping-like phenomenon [16] due to a low stability constant and a high dissociation rate. Without a large excess of lactate, the stripped iron ions are exposed to a strong interaction with gel matrix and thus retained tightly in the column.

The paper electrophoresis of the iron-containing fraction of gel filtration revealed the presence of Fe^{3+}–citrate complex as a minor component. Citrate was shown by Sarkar [17] to be the major Fe^{3+}-binding substance in the small molecular weight fraction of human serum. It was also found [18] that citrate prevented attachment of non protein-bound iron to the gel but did not affect protein binding. However, its contribution on an iron flux into the fetal circulation may be very small.

It has been shown in several reports [19–24] that, in developing erythroid cells, polyphosphate compounds such as ATP, GTP and 2,3-diphosphoglycerate may play an important role in iron release

from circulating transferrin and in the intermediate functional iron pool for metabolic needs. Such a mechanism may possibly operate in placental cells. The concentration of these compounds in the placental ultrafiltrate is too low to be demonstrable on the ^{13}C NMR spectrum.

Iron in the term placenta is mainly deposited just beneath the trophoblastic epithelium, although it is demonstrable in considerable quantities in the stroma of the chorionic villi in the first half of gestation [25]. This alteration is understood as a process which should increase the ease of transfer. With advancing gestation, the placenta changes from an organ of active to one of passive filtration. An overwhelming majority of lactate in the placenta may function as a safeguard against the precipitation of iron hydroxide and as a major ligand in the intermediate transit iron pool.

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