THE ISO-ELECTRIC FRACTIONATION AND PROPERTIES OF RABBIT TRANSFERRIN

H.G.VAN EYK, R.J.VERMAAT and B.LEIJNSE
Department of Chemical Pathology, Medical Faculty Rotterdam, Rotterdam, The Netherlands

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1. Introduction

The isolation and characterization of rabbit transferrin has been described [1, 2]. Transferrin is the principal iron binding component of serum. In humans and in rabbits, it exists in a partially unsaturated state. We have observed that on starch-gel electrophoresis and polyacryl amide-gel electrophoresis purified preparations of rabbit (and also human) transferrin show two components and this raises the question of the identity of the transferrin-iron complex in the body [3]. The results of the present work support the view that transferrin appears in three molecular species, corresponding to the iron free protein, the one iron mol-protein complex and the two iron mol-protein complex.

2. Materials and methods

2.1. *Reagents*

The chemicals used were of analytical reagent grade.

2.2. *Starch-gel electrophoresis*

Polyacrylamide-gel electrophoresis and immunoelectrophoresis were carried out as described earlier [1].

2.3. *Preparation of proteins*

Rabbit transferrin was prepared from rabbit serum as described in an earlier publication [1]. Iron free proteins were prepared by the method of Bezkorovainy [7], half and fully saturated proteins were prepared by the method of Ramsay [8]. The iron containing proteins were labelled by addition of 5–10 μCurie $^{59}FeCl_3$.

2.4. *Determination of iron*

To transferrin solutions, after reduction with $Na_2S_2O_3$, bathophenantholine was added, and the pink colored complex with Fe^{2+} was extracted with methyl isobutyl ketone. In this extract the Fe content was determined by atomic absorption spectrophotometry [4]. Iron-59 was counted with a Philips gamma counting apparatus PW 4025–PW 4280.

2.5. *Iso-electric fractionation*

For iso-electric fractionation, the LKB 8101 Ampholine Electrofocussing Equipment was used (with a column of 110 ml). An ampholyte solution with pH range 5–7 was used. Running tap water was circulated round the column. Electrode solutions were 1% sulfuric acid and 8% sodium hydroxide. The current was passed through the column for 40 hr; for the last 20 hr the voltage was 500 V and the power supply never exceeded 1 Watt. After 40 hr the column was drained at about approx. 1 ml/min. Fractions of 1 ml were collected. The protein content was monitored at 254 nm continuously, determined from the E 280 value, and by the Lowry method.

3. Results

The iso-electric fractionation of either a mixture

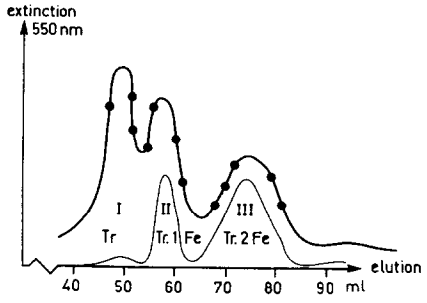

Fig. 1. Iso-electric fractionation of rabbit transferrin preparations.

of 20 mg apo-transferrin (T_0) and 20 mg fully saturated transferrin (Tr 2Fe) or of a rabbit transferrin preparation of 40 mg that had been approximately half-saturated with iron revealed three protein fractions (fig. 1).

The first fraction was nearly colourless while fractions 2 and 3 were visible as pink bands before the elution had taken place. The pH was measured in the three tubes containing solutions with the highest extinction at 280 nm. The iso-electric points, uncorrected for the presence of sucrose were in peaks 1, 2 and 3, respectively 6.0, 5.5 and 5.1. In fraction no. 1 no iron could be detected, in fraction no. 2 the protein iron ratio expressed in moles was 1:1 and in fraction no. 3, 1:2. Thus in preparations half saturated with iron three molecular species exist. During the electrofocussing procedure of a mixture of apo-transferrin and fully saturated transferrin the 1 mol iron-protein complex is formed. The 1 mol iron-protein component was collected from several experiments, and subjected to the same electrofocussing procedure. Three components were again obtained indicating that during the time of electrophoresis an exchange of iron occurs.

4. Discussion

The experiments have shown that a rabbit transferrin preparation, in a partially unsaturated state, exists as three molecular species, the apo-transferrin and the one mole iron-protein and the two mol iron-protein complex. The results agree with those of Wenn and Williams [3] described for ovo transferrin. This means that iron binding is not an all or none phenomenon [5]. The iron must be bound in the following steps:

$$\text{apo-transferrin} + \text{Fe} \overset{K_I}{\rightleftarrows} \text{transferrin Fe}$$

$$\text{transferrin Fe} + \text{Fe} \underset{K_{II}}{\leftrightarrows} \text{transferrin 2 Fe}$$

Equilibrium dialysis studies from which the equilibrium constants K_I and K_{II} could be derived have proved this two step process [6].

5. Summary

When non-saturating amounts of iron are added to rabbit apo-transferrin solutions, the preparations can be separated into three molecular species by the electrofocussing technique in an ampholyte solution pH 5–7. The existence of three components, apo-transferrin, one iron-mol-protein and two iron-mol-protein complex have been proved.

These findings support the view that iron is bound to rabbit apo-transferrin in two steps.

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