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Isolation of an Iron-Containing Red Protein from Human Milk

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The presence of a red protein in bovine milk has been observed by several authors [1-4]. Sörensen and Sörensen [1] as well as Polis and Shmukler [3] performed a partial purification of this protein and recently a procedure for its isolation was described by Grooves [4]. The protein isolated by Grooves contained iron and was in certain respects similar to the iron-binding protein in blood plasma (transferrin [5] or β_1-metal-combining protein [6]). According to Schäfer [7] *human* milk assumes a red color after addition of small amounts of iron, probably due to the formation of an iron-protein complex. It has also been observed in this laboratory that crude preparations of human milk whey proteins are often salmon coloured[8]. This colour becomes more pronounced after addition of iron to the milk. A partial purification of the red component has been achieved by chromatography on calcium phosphate [9]. A method for isolation of the red protein has now been worked out and will be presented below.

Four litres of fresh human milk were defatted by centrifugation. After addition of 10 mg of Fe^{2+} (in the form of ferrous ammonium sulphate) to the milk, giving it a distinct reddish tint, solid ammonium sulphate was added to 45 % saturation and pH adjusted to 8.0 by careful addition of concentrated ammonia. When the milk was warmed to 37° for 30 min a flocculent precipitate was formed, which was removed by filtration through a fluted paper (Whatman No. 12). The rate of filtration was most often slow and the filtration had to proceed overnight at +4°C. The precipitate was discarded and the red-brown filtrate was acidified to pH 4.0 with 1 M sulfuric acid. The precipitate formed was quickly removed by gravity filtration or better by centrifugation and the pH of the filtrate (supernatant) immediately readjusted to 8.0. Ammonium sulphate was added to 80 % saturation and the red precipitate collected by filtration and dissolved in a minimal amount of distilled water. The dark red solution was dialyzed against distilled water followed by dialysis against 0.02 M sodium phosphate buffer, pH 8.0. The solution was then chromatographed on DEAE-cellulose [10] previously equilibrated with the same buffer. Under the conditions described the red protein was not sorbed on the ion-exchanger but appeared in the effluent without significant retardation. The red fraction was further chromatographed on calcium phosphate as described earlier [9], using phosphate buffers of pH 8.0 instead of 6.9. The red fraction eluted with 0.5 M phosphate buffer was dialyzed against distilled water and finally

Fig. 1. Electrophoretic pattern of the purified red protein. Glycine-NaOH buffer, pH 9.4, ionic strength 0.1. Ascending boundaries to the left, descending boundaries to the right. Duration 350 min.

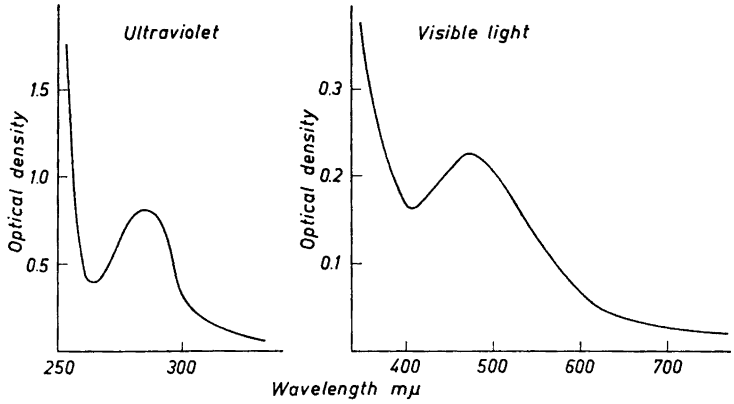

Fig. 2. Absorption spectrum of the red protein at pH 9.0.

precipitated with ammonium sulphate between 60 and 75 % saturation at pH 8.0.

The product obtained was run in moving boundary electrophoresis and found to be homogeneous in glycine-NaOH buffer of pH 9.4 and ionic strength 0.1 (Fig. 1) and also in sodium veronal-HCl buffer of pH 8.6 and ionic strength 0.1. Electrophoresis at pH 8.0, however, showed an asymmetric peak on the ascending limb that might be due to a heterogeneity of the preparation.

Analysis of the iron content according to Lorber [11] after combustion with sulfuric acid and 30 % hydrogen peroxide [12] showed the presence of 0.13—0.15 % iron in different preparations. After passage of the protein through a column with cation exchange resin (Dowex 50, X-12) at pH 8.5 the iron content was diminished to 0.10—0.11 %. The main part of the iron could thus not be removed by the resin. The light absorption was examined in the wavelength range of 225—700 mμ (Fig. 2) in a Zeiss spectrophotometer, PMQ II, using 1 cm cuvettes. In the ultraviolet region only the expected maximum around 280 mμ was found. In the region of visible light a broad maximum at 460—470 mμ and a minimum around 410 mμ were found. At a protein concentration of 1.0 % (pH 9.0) the optical density at 460 mμ varied between 0.40 and 0.45 for different preparations (1 cm light pathway). The ratio of the optical densities at 460 to 408 mμ varied between 1.30 and 1.38. The type of light absorption curves and the presence of

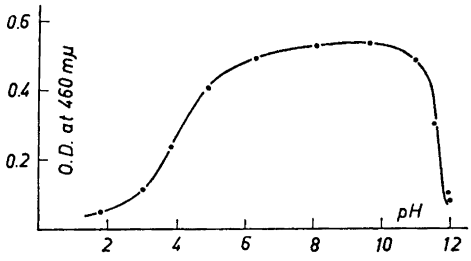

Fig. 3. The pH-dependance of the light absorption of the red protein at 460 mμ.

strongly bound iron suggests a certain similarity to blood serum transferrin. The milk protein is, however, not identical to transferrin, as there is no immunological relationship between the two proteins as determined by diffusion-in-gel analysis [13]. Furthermore the iron complex formed by the milk protein seems to be more stable in acid media than the iron-transferrin complex. The milk protein is thus not completely decolorized until pH 2 (Fig. 3) and at this pH 90—95 % of the iron is removable by passing the protein through a column of Dowex 50, X-12.

These and further studies will be reported in detail shortly.

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Perjodatoxydation von Phenolen VI*. Dimere *o*-Chinole aus Mesitol und 2,6-Dimethylphenol

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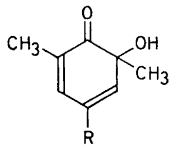
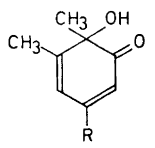
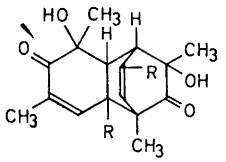
Die Einwirkung von Natriumperjodat auf wässrige Lösungen von Mesitol bzw. 2,6-Dimethylphenol führt in rascher Reaktion zu Substanzen vom Schmp. 183° bzw. 196°, für welche die Strukturen der Dimeren V[1] bzw. VI angenommen wurden. Ihre Entstehung war verständlich mit der Annahme, dass die Phenole zunächst zu den *o*-Chinolen I bzw. II oxydiert werden, und dass die letzteren sich durch Diels-Alder-Reaktion dimerisieren. Wessely und Mitarb. hatten das Dimere vom Schmp. 183° auch bei der alkalischen Verseifung von 2,4,6-Trimethyl-*o*-chinolacetat (I, OAc statt OH) erhalten und für das Produkt ebenfalls Struktur V vermutet [2]. Direkter Vergleich der beiden Präparate bestätigte deren Identität.



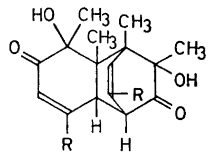
I. R = CH_3
II. R = H



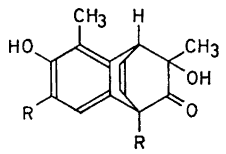
III. R = CH_3
IV. R = H



V. R = CH_3
VI. R = H



VII. R = CH_3
VIII. R = H



IX. R = CH_3
X. R = H

Kürzlich berichteten jedoch Wessely und Mitarb.[3] über Versuche, welche die genannten Autoren zu einer Revision der Formel V veranlasste. Man fand, dass die alkalische Verseifung von 2,4,6-Trimethyl-*o*-chinolacetat (I, OAc statt OH) neben dem Dimeren vom Schmp. 183° überraschenderweise monomeres 2,3,5-Trimethyl-*o*-chinol (III) lieferte. Im alkalischen Medium war demnach eine Acyloinumlagerung einge-

* V. Mitt.: Adler, E., Junghahn, L., Lindberg, U., Berggren, B. und Westin, G. *Acta Chem. Scand.* **14** (1960). *Im Druck.*