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IMMUNOCHEMICAL IDENTIFICATION OF FERRITIN AND ITS IMMUNOLOGIC ANALOGS β-FETOPROTEIN AND α_{2H}-GLOBULIN

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During the last 15 years several proteins possessing a common antigenic determinant with the ferritin of the liver have been described: a tissue α_2-globulin, β-fetoprotein, and α_{2H}-globulin [3, 6, 9]. The tissue α_2-globulin, isolated by me from human kidney tumor tissue, was indistinguishable from ferritin, and in later publications I called it ferritin [4]. The β-fetoprotein, isolated by Alpert et al., from human embryonic liver, had the electrophoretic mobility of blood serum β-globulins (ferritin has the electrophoretic mobility of α_2-globulin) and, despite its immunologic identity with the ferritin of the liver, it was classed by these workers among the human fetoproteins [6]. The α_{2H}-globulin isolated by Buff et al. from tumor tissues was very similar in its properties to ferritin but, in addition to its iron-containing fractions, it had an additional glycoprotein component which is not present in ferritin preparations [10]. This difference, these workers consider, did not allow this protein to be called ferritin [10]. As a result, some ambiguity has arisen in the terminology of ferritin in the literature, and some workers have expressed the wish either to describe the "newly discovered ferroproteins" immunologically identical with ferritin as ferritin or to argue strongly in support of giving this protein a name of its own [12].

The immunodepressive properties of ferritin [8] and the carcinogenic effect of iron on human and animal cells [11, 14] are the reasons for the undiminishing interest in ferritin, the iron depot protein in the body [7, 12, 13]. Hence the need for clear differentiation not only between the various ferroprotins, but also between the different types of ferritins, for example the isoferritins isolated from normal and tumor tissues. The object of the present investigation was to identify ferritin and its immunologic analogs described in the literature under different names.

EXPERIMENTAL METHOD

Ferritin was crystallized from normal, tumor, and embryonic tissues by Granick's method. α_{2H}-globulin was isolated by the method of Buff et al. [10]. The purity of the preparations was verified by disc electrophoresis and disc immunoelectrophoresis [2]. A preparative version of disc electrophoresis in polyacrylamide gel, the methods of immunoelectrophoresis and gel filtration in [1], and the immunodiffusion method and methods of specific detection of glycoproteins and ferroproteins in [4] also were used.

Antiferrin sera were obtained by immunizing rabbits: 1 mg ferritin with Freund's adjuvant was injected subcutaneously once a week for 4 weeks. Blood was taken on the 7th day. The antiferritin sera did not reveal antibodies against other serum and tissue proteins and they contained about 0.5 mg/ml of antiferritin antibodies. Antisera against the glycoprotein component of α_{2H}-globulin and against α_{2H}-globulin itself were obtained by the same scheme, but they contained antibodies both against the glycoprotein components and against ferritin.

The monospecific antiserum against β-fetoprotein was obtained from Alpert (USA) in 1975 and that against α_{2H}-globulin from Burtin (France) in 1973. Ferritin and β-fetoprotein were

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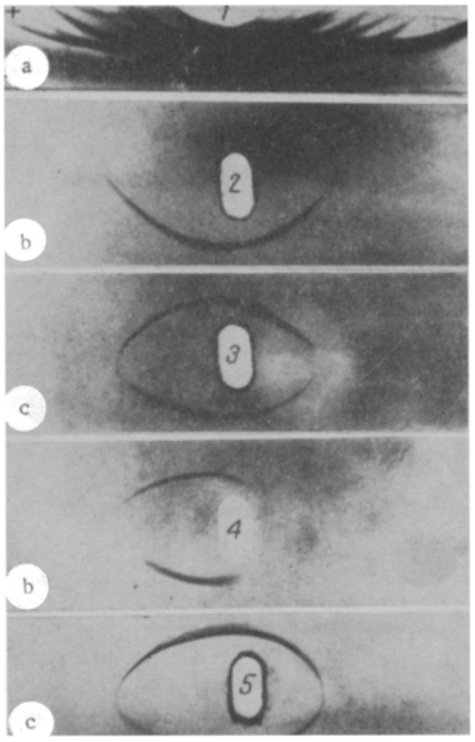

Fig. 1. Immunoelectrophoretic analysis of ferritin and β-fetoprotein. 1) Human blood serum; 2, 3) ferritin from embryonic liver; 4) ferritin from kidney tumor; 5) ferritin from hematoma. a) Antiserum against human plasma proteins; b) antiserum against β-fetoprotein (Alpert, USA); c) antiferritin serum.

identified by immunoelectrophoresis and by comparison of standard test systems for these proteins in the precipitation test. To identify ferritin and α_{2H}-globulin, we used the precipitation test (comparison of test systems), disc electrophoresis with double staining of the columns of the gels for glycoproteins and ferroproteins, preparative isolation of α_{2H}-globulin and ferritin subfractions, preparation of antisera against the different subfractions of these proteins, and comparison of test systems for these subfractions.

EXPERIMENTAL RESULTS

The results of a comparative immunoelectrophoretic study of ferritin and β-fetoprotein are illustrated in Fig. 1. Ferritin from embryonic and definitive human liver served as the antigens. Migration of ferritins, revealed by antiferritin serum and antiserum against β-fetoprotein, was identical and corresponded to the α_2-globulin zone. During identification of ferritin, β-fetoprotein, and α_{2H}-globulin by comparison with one another in the precipitation test they were found to be immunologically completely identical (Fig. 2a).

The disc-electrophoretic pattern of ferritin and α_{2H}-globulin from a kidney tumor is shown in Fig. 2b. The ferritin of the liver is represented by three fractions: fraction A — monomer, subfraction B — dimer, and subfraction C — polymer. The molecular weight of the main fraction of ferritin, the monomer A, on Sephadex G-200 varied depending on the origin of the ferritin from 440,000 to 480,000. All three ferritin fractions from the liver stained for protein and fetoprotein in polyacrylamide gel. All three fractions stained for protein in the preparation of α_{2H}-globulin from the kidney tumor, but only two stained for ferroproteins: A and B (Fig. 2b). Double staining of the columns after disc electrophoresis for glycoproteins and ferroproteins showed that subfraction C of α_{2H}-globulin is a glycoprotein.

Under similar conditions the ferritin preparations from embryonic, definitive, and neoplastic liver tissue, definitive and neoplastic kidney tissue, and definitive spleen tissue were stained, but no glycoprotein component could be discovered in these preparations. Characteristic features of ferritins for different organs and tissues, clearly distinguishable

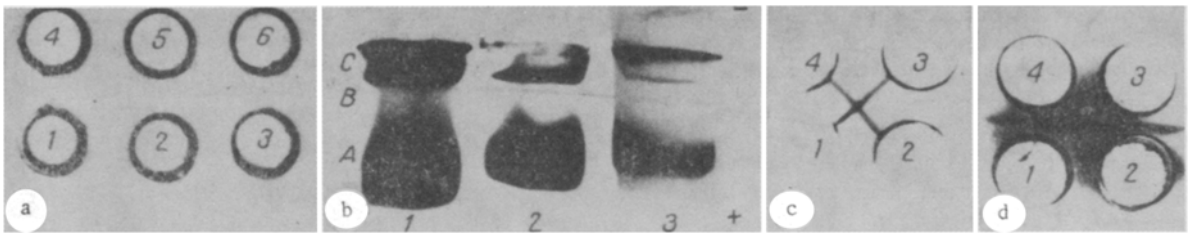

Fig. 2. Immunochemical identification of ferritin, β-fetoprotein, and α_{2H}-globulin. a) Comparison of test systems for ferritin, β-fetoprotein, and α_{2H}-globulin: 1) antiferritin serum; 2) antiserum against α_{2H}-globulin (from Burtin, France); 3) antiserum against β-fetoprotein (from Alpert, USA); 4) α_{2H}-globulin from kidney tumor; 5) liver ferritin; 6) embryonic liver ferritin; b) disc-electrophoretic study of liver ferritin and α_{2H}-globulin from kidney tumor: 1) liver ferritin (stained for ferroproteins); 2) α_{2H}-globulin (stained for ferroproteins); 3) α_{2H}-globulin (stained for proteins); c) comparison of test systems for ferritin and glycoprotein component of α_{2H}-globulin from kidney tumor: 1) glycoprotein component of α_{2H}-globulin 2) ferritin from kidney tumor (fraction A); 3) antiserum against glycoprotein component of α_{2H}-globulin; 4) antiserum against fraction A of ferritin from kidney tumor; d) identification of glycoprotein component of α_{2H}-globulin; 1) antiserum against glycoprotein component of α_{2H}-globulin; 2) antiserum against α_2-macroglobulin (from Behringwerke, West Germany); 3) α_{2H}-globulin from kidney tumor; 4) α_2-macroglobulin (from Behringwerke, West Germany)

on electrophoresis in polyacrylamide gel, must be noted. They apply primarily to the number of subfractions in the ferritin preparations and the form and presence of additional iron-containing components of the monomer fraction — anodal or cathodal "loops."

After electrophoresis in polyacrylamide gel subfractions A, B, and C of α_{2H}-globulin and ferritin were obtained preparatively and used to immunize rabbits. Adsorption of antiserum against the glycoprotein component of α_{2H}-globulin (subfraction C) of the monomer A fraction of α_{2H}-globulin or of any ferritin fraction had no effect on activity of the antiserum; its absorption by subfraction C of α_{2H}-globulin or donor's blood serum, however, inactivated the antiserum. Adsorption of antiserum against the monomer A fraction of α_{2H}-globulin with the glycoprotein component of α_{2H}-globulin (subfraction C) or donors' blood serum did not affect activity of the antiserum; its absorption with the ferroprotein component of α_{2H}-globulin or any fraction of liver ferritin inactivated the antiserum.

Immunochemical identification of the glycoprotein and ferroprotein components of α_{2H}-globulin showed them to be completely unidentical (Fig. 2c). This glycoprotein component of α_{2H}-globulin was shown to be a blood serum protein, namely one of the α_2-macroglobulins of blood serum (Fig. 2d).

Consequently, the glycoprotein component of α_{2H}-globulin is the basis for differences between ferritin and α_{2H}-globulin: It is not identical to ferritin immunochemically and it is not a component of the tumor and embryonic ferritins. The present writer is inclined to regard its appearance in tumor tissues as the result of a change in the classical method of isolation of ferritin. The difficulty of isolating ferritin from tumor tissues and, in particular, from kidney tumor tissue, must be emphasized: The recrystallized ferritin preparation is only sparingly soluble in 0.14 M sodium chloride solution. As a result of this, much protein is lost. An increase in the ionic strength of the dialyzing solution, according to the method of Buff et al. [10], gives a better yield of ferritin, but in this case the glycoprotein serum component passes into solution.

The β-fetoprotein and α_{2H}-globulin described in the literature are thus immunologically identical with ferritin, and it is the writer's opinion that both β-fetoprotein and α_{2H}-globulin ought to be called ferritin.

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