The serological specificity of chicken hemoglobin fractions

By V. D’AMELIO and A. M. SALVO

Laboratory of Comparative Anatomy, The University of Palermo


Three fractions are separable from chicken Hb, using electrophoresis on starch. Agar plate double diffusion tests and immunoelectrophoresis indicate that two of them have the same serological specificity.

The third one, i.e. the slowest moving one, has specific serological characters.

Several reports have been published on the electrophoretical behaviour of animal hemoglobins (Hb) (see references 1—5). On the contrary, there are relatively few immunological studies on Hb and these mostly limited to human Hb.

Having undertaken a serological investigation on the ontogenesis of Hb in the chicken embryo, it seemed necessary to obtain further information as to the serological specificity of the fractions present in the Hb of the adult animals.

In the adult chicken, in fact, two or three components have been identified by paper electrophoresis 3—5. By chromatography on starch column, two components were separated from chicken Hb differing in the relative amount of acid and basic amino acids. This observation suggested the possibility of the existence of differences in the immunological determinant groups of the fractions concerned.

By applying the double diffusion technique of Ouchterlony 8 and the immunoelectrophoretic technique of Grarab and Williams 9 we have succeeded in showing two different serological types among the three fractions separated electrophoretically from Hb of adult chicken.

Material and methods

The Hb has been prepared from the red cells of adult White Leghorn and New Hampshire chickens, and from reticulocytes of anemic chickens following the technique of Drabkin 10. The red cells were washed in saline, hemolyzed in distilled water overnight in the refrigerator and the cell debris removed by centrifugation at 30 000 g for 60 minutes in the cold. The Hb was crystallized by dialysis against 2.8 M phosphate buffer at pH 6.8, washed with the same buffer and redissolved with the original amount of distilled water. The crystallization was repeated at least twice.

Antisera were obtained after two injections of 1 ml of concentrated Hb mixed v/v with Freund adjuvant 11. The second injection was made three weeks after the first one, and the sera were collected three weeks later. Eight rabbits were used and the sera tested separately. For preparative purposes the electrophoretic separation of the Hb fractions was carried out on starch, following essentially the method of Kunkel 12, using either 0.1 M barbiturate or 0.1 M borate buffer both at pH 8.6.

375 gr. of starch were mixed with a convenient amount of buffer and poured on a glass plate 20·35 cm. and the excess liquid removed by blotting the cake with filter paper. Five samples of concentrated Hb, of 0.1—0.5 ml each, were distributed on the same plate. The electrodes were immersed in vessels containing double concentrated buffer. A potential difference of 10 volts/cm. for 40 hours was applied when barbiturate buffer was employed and of 11 volts/cm. for 6 hours in the case of borate buffer.

Immunoelectrophoresis and electrophoresis on agar gel 1.5% were run as described by Grarab and Williams using a veronal buffer 0.05 M at pH 8.6·10 ml of 3% agar were mixed v/v with 0.1 M veronal buffer and poured on a glass plate 5·30 cm. A potential difference of 8 volts/cm. was applied for 15 hours. Electrophoresis were always carried out in the cold.

Results

Chicken Hb, prepared both from normal red cells or reticulocytes, when submitted to electrophoresis

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1 H. A. Itano, Annu. Rev. Biochem. 25, 331 [1956].
2 H. A. Itano, Advances Protein Chem. XII, 215 [1957].

10 D. L. Drabkin, Arch. Biochemistry 21, 224 [1949].
on starch, separated into three components all with cathodic migration when borate buffer was used (Fig. 1). We shall refer to them as component C1.

C2 and C3 in order of decreasing mobility. In most cases only two components separate with veronal buffer; occasionally, a third component with anodic migration appears (Fig. 2).

With electrophoresis on agar, only two components appear; the fastest one, however, exhibits considerable tailing. In the immunoelectrophoretic tests the two components give rise to precipitation lines which join together. On the other hand one or more precipitation lines appear in conjunction with the “tail” which do not bear any relation to the others.

As it will be discussed below, the agar plate diffusion technique gives evidence of a serological specificity of component C3; hence, it would be suggested that the precipitation lines appearing in conjunction with the “tail” may be due to the presence in it of component C3.

The fact that precipitation lines appear only in connection with the Hb spots suggests that antibodies against proteins other than Hb, even if present in our antisera, do not play any role in these reactions. A contamination with nucleic acids seems hardly avoidable in preparations of Hb from avian red cells. The spectrophotometric analysis indicates, in fact, that such slight contamination is present in our preparation.

In the serological experiments with double diffusion technique on agar plates (Ouchterlony technique) total Hb and the three Hb fractions, isolated with starch electrophoresis, were tested against anti-total Hb sera.

A thick and serologically identical line of precipitation is produced by all antigens (Fig. 3 – 3 bis). In some experiments, presumably due to a better electrophoretical separation, fraction C3 failed to produce this line (Fig. 4 – 4 bis).

Sometimes C2 and C3 give rise instead of one single thick line to a number of thinner lines which all join the thick line produced by total Hb or C1. It is to be noted, however, that these two fractions have always been used more diluted than C1, or total Hb.

In addition, two or three thinner lines, which usually migrate slower than the thick line, are produced by total Hb and C2 and C3 components. They are always apparent even when C3 fails to give rise to the thick line. C1, on the contrary, usually does not cause the appearance of these thin lines, or when it does, they are located very close to the antigen well thus indicating the presence of only a very small amount of the related antigen. Slight differences in these patterns have been observed with some of the tested antisera.

These results seem thus to suggest a serological

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difference among the electrophoretically separated Hb fractions or, at least, between C.3 and the others. Repeated crystallization does not modify this pattern.

The fact that with both serological techniques used in these experiments each one of the electrophoretically separated fractions gives rise to a number of precipitation lines, could be taken as evidence of a microheterogeneity among Hb fractions. However, the possibility of formation of different antibodies against different determinant groups reacting at different equivalent point; the formation of aggregates during the preparation of the antigen and/or the incubation procedure and, finally, dilution effects\textsuperscript{14–15} should also be taken into consideration.

Experiments on Hb fractionation are now in progress with electrophotographic technique.

\textsuperscript{14} J. S. \textsc{salvini} and M. K. \textsc{aminski}, C. R. \textsc{séances Soc. Biol. Filiales} 240, 377 [1955].
\textsuperscript{15} R. K. \textsc{jennings}, J. Immunology 77, 156 [1956].

\textbf{Saccharomyces} als Objekt für populations-genetische Versuche

\textsc{von H. Gutz}\textsuperscript{*} und C. C. \textsc{emis}

\textit{Aus der mikrobiologischen Abteilung des Instituts für Gärungsgewerbe, Berlin N 65 (Leiter: Prof. Dr. S. \textsc{windisch})}

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Microorganisms are suitable objects for experimental studies in population genetics. It is easy to cultivate large populations under exactly controlled conditions. But microorganisms reproduce usually vegetatively so that one factor is lacking which is of great importance in populations of higher organisms: The sexual propagation which implies a permanent rearrangement of the genes present.

\textit{Saccharomyces} is an unicellular ascomycet. The cells are diploid and propagate vegetatively by budding. Under certain conditions \textit{Saccharomyces} is able to reproduce sexually by formation of ascospores. We have developed a simple technique which allows to enforce exclusively a sexual propagation in a yeast. Thus \textit{Saccharomyces} is a suitable object for experiments in population genetics, in which the rearrangement of genes by sexuality has to be considered. The existence of a diploid phase makes it possible to work on problems resembling those in populations of higher organisms.

In the present work methods are described for experimental work with yeast populations, and possibilities are which \textit{Saccharomyces} offers for experiments in population genetics are discussed.

Unsere Vorstellungen über genetische Veränderungen in Populationen beruhen vorwiegend auf theoretischen Überlegungen; eine ausführliche Darstellung ist in dem Buch von \textsc{Lt} \textsuperscript{1} zu finden. Eine experimentelle Nachprüfung dieser Vorstellungen ist mit höheren Pflanzen oder Tieren nur in begrenztem Rahmen möglich, da sie lange Generationszeiten haben und nicht eine beliebig große Anzahl von Individuen verwendet werden kann. Ferner ist es schwierig, die Umweltbedingungen konstant zu halten. Im Gegensatz zu höheren Organismen können Mikroorganismen unter genau kontrollierbaren Bedingungen und in kurzer Zeit zu beliebig großen Mengen herangezüchtet werden. So sind z. B. in einer ausgewachsenen Hefekultur etwa $10^7$ Zellen pro ml vorhanden. Wegen der großen Zahl von Individuen und der schnellen Vermehrung sind Mikroben vor allem für Untersuchungen über Mutatio- nen und Selektionsverhältnisse geeignet. Viele derartige Versuche sind mit Bakterien gemacht worden \textsuperscript{2}.

Mikroorganismen scheinen daher sehr geeignete Objekte für populations-genetische Versuche zu sein. Sie vermehren sich jedoch in der Regel ausschließlich vegetativ, so daß ein Faktor fehlt, der in Populationen höherer Organismen von großer Bedeutung ist: Die sexuelle Fortpflanzung und die damit verbundene Umkombination des Erbgutes. \textit{Saccharomyces} ist nun ein einzelliger Ascomycet, in dessen Lebenszyklus eine sexuelle Vermehrung auftritt. Wegen der Fähigkeit zur sexuellen Fortpflanzung ist diese Hefe für populations-genetische Versuche geeignet, in denen die durch die Sexualität bedingte Umkombination des Erbgutes berücksichtigt werden soll.

\textsuperscript{*} z. \textsc{Lt. Institut für allgemeine Botanik der Universität, Zürich.}

\textsuperscript{1} C. C. \textsc{Lt}, Population genetics. 2. edit., Chicago 1958.