Hybridization of Glucose-6-Phosphate Dehydrogenase from Rat and Human Erythrocytes

Abstract. *Glucose-6-phosphate dehydrogenase from rats and from humans was partially purified and was inactivated (dissociated) by removing triphosphopyridine nucleotide by repeated washing and dialysis, and then it was reactivated by addition of triphosphopyridine nucleotide and incubation at 25°C. When enzyme from each species was mixed with enzyme from the other during or after inactivation, a new electrophoretic band located between the fast-moving rat dehydrogenase and the slow-moving type B human enzyme appeared. This band is interpreted as representing a hybrid composed of polypeptide chains from each enzyme.*

It has become increasingly apparent that many proteins may exist as polymers of different polypeptide chains. Under suitable experimental conditions such chains may be dissociated and then reassociated. When dissociated polypeptide chains from different sources are mixed and then allowed to reassociate, "hybrid" molecules may be formed which contain polypeptide chains from each of the two mixed molecular forms. Examination of these hybrid forms may provide information regarding the polypeptide chains that take part in a mutation. Hybridization of human with dog hemoglobins has been used for this purpose (*1*). To our knowledge, hybridization of enzymes from different animal species has been carried out only with rabbit and mouse lactate dehydrogenase (*2*).

In 1962 Kirkman and Hendrickson (*3*) provided evidence that glucose-6-phosphate dehydrogenase (G-6-PD) occurred as a dimer which could be dissociated by the removal of triphosphopyridine nucleotide (TPN) and then could be reassociated by addition of triphosphopyridine. Chung and Langdon (*4*) confirmed these results and established, by analysis of terminal amino acids, that at least two different amino acid chains are involved. A possible hybrid G-6-PD occurring in vivo in *Peromyscus* has recently been reported (*5*). We now report production of an artificial G-6-PD hybrid, one between enzyme derived from erythrocytes of Wistar rats and enzyme from human red cells, electrophoretic type B.

Glucose-6-phosphate dehydrogenase was partially purified by the method of Kirkman (*6*). The enzyme was precipitated with ammonium sulfate, and TPN was removed by washing several times with ammonium sulfate in a tris buffer containing ethylenediaminetetraacetic acid (EDTA) and mercaptoethanol. The enzyme was then redissolved in tris buffer containing EDTA and mercaptoethanol and dialyzed against this mixture for 18 hours. The enzymes from each species were dialyzed separately and together. Dialyzed enzyme was reactivated by addition of TPN to give a final concentration of $10^{-5}M$ and by incubation at 25°C. Before and after 8 hours of incubation, appropriately diluted samples were subjected to starch-gel vertical electrophoresis (*7*). The conditions of electrophoresis and the development of the enzyme pattern (*8*) were modified only by addition of a small amount of catalase to the staining mixture to retard nonspecific reduction of dye. Storage of the reactivated mixed or unmixed samples at 4°C for several days produced no change in the electrophoretic pattern.

Figure 1 shows the electrophoretic patterns obtained when samples of human enzyme and rat enzyme were inactivated (dissociated) separately and together and were reactivated separately and together. The reactivated enzymes were each represented by a sharp band with a mobility essentially identical with that of purified or crude enzyme. Mixtures of reactivated enzyme separated clearly into two bands having the same mobility as native rat and human enzyme. Rat and human enzyme which had been mixed before reactivation showed, in addition, the presence of a third band, presumably the band of a hybrid, intermediate in position between the bands of the rat enzyme and the slow-moving human enzyme. The third band was particularly pronounced when the enzyme was mixed before dialysis; it could also be demonstrated prior to reactivation. Since the method of enzyme detection depends upon the biochemical activity of the enzyme, it is apparent that the hybrid enzyme retained the capacity to oxidize G-6-PD and reduce TPN.

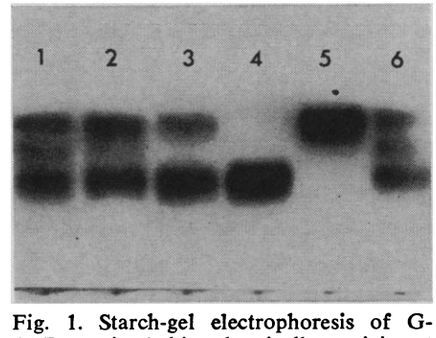

Fig. 1. Starch-gel electrophoresis of G-6-PD, stained histochemically; origin at bottom; highest potential (+) at top. Each slot received approximately the same amount of enzyme, which had been inactivated and reactivated; approximately 57 percent of the human enzyme and 44 percent of the rat enzyme were inactivated at the end of dialysis. Reactivation was complete. Channels 1 and 6: rat and human enzyme mixed prior to dialysis during the inactivation procedure. Channel 2: rat and human enzymes mixed prior to reactivation. Channel 3: rat and human enzymes mixed after reactivation with TPN. Channel 4: human enzyme. Channel 5: rat enzyme.

Previously we attempted to hybridize glucose-6-phosphate dehydrogenase from three types of enzyme-deficient donors, those with the African and Mediterranean types of deficiencies and those whose deficiency is associated with nonspherocytic congenital hemolytic anemia. If the mutation resulting in loss of activity were a structural one affecting different polypeptide chains in the different types of G-6-PD deficiency, a hybridization of two inactive enzymes might have resulted in the formation of active enzyme. The results of these early studies were negative (*9*).

Mixing dissociated human and rat G-6-PD resulted in appearance of a new electrophoretic band midway between the original bands. The new band was particularly pronounced when the partially inactivated preparations were dialyzed in the absence of TPN. It therefore seems probable that dissociation and reassociation occurred during this proces . In contrast, storing mixed, reactivated enzyme at 4°C in the presence of $10^{-5}M$ TPN failed to result in the formation of hybrid enzyme. The dissociation and subsequent reassociation of two dissimilar dimers could give rise to four dimers. Thus, if the human enzyme is arbitrarily named $\alpha^h\epsilon^h$ and the rat enzyme $\alpha^r\epsilon^r$, then the following four enzymes could be expected on hybridization, $\alpha^h\epsilon^h$, $\alpha^h\epsilon^r$, $\alpha^r\epsilon^h$, and $\alpha^r\epsilon^r$. Four bands were not found, however. This may be due to identical mobility of the two hybrid bands $\alpha^r\epsilon^h$ and $\alpha^h\epsilon^r$, or to failure of one of the two hybrid species to form because of some incompatibility in their polypeptide structures.

It may be possible to form other G-6-PD hybrids. Rat and type B human enzyme were chosen for hybridization in these studies because rat enzyme appeared to have the most rapid mobility among those of species readily available (mouse, rat, cow, sheep, rabbit, coyote). Thus, there was a well-marked difference in the mobility of the two enzymes. Cow enzyme had the least electrophoretic mobility, but attempts to hybridize this enzyme with the rapidly moving human type A enzyme have failed.

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References and Notes

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Submaxillary Gland of Mouse: Effects of a Fraction on Tissues of Mesodermal Origin in vitro

Abstract. *A macromolecular fraction from mouse salivary glands markedly enhances growth of mesenchymal tissues in vitro. The same fraction causes dedifferentiation of tissues of mesodermal origin, such as muscle and cartilage. Dedifferentiated tissues do not divide but survive in good condition, while controls in the same cultural media undergo regressive changes.*

Mouse salivary gland contains several factors endowed with unique and remarkable biologic properties. The most striking factor is NGF, a protein that specifically stimulates growth of sympathetic nerve cells in vitro and in vivo; we have extensively studied its chemical and biologic properties (*1*).

Subsequently another growth-promoting factor of a protein nature, EGF, specific for epidermal cells, has been isolated from extract of mouse salivary gland and purified (*2*). Another fraction of homogenate of the same gland is under investigation regarding its ability to induce intense leukocytosis when minute amounts are injected into various animals.

We now report certain effects of still another macromolecular fraction of salivary gland on cultures of embryonic tissues of mesodermal origin; this fraction stimulates the growth of mesenchymal cells and causes loss of differentiative marks in muscle and cartilage.

The active fraction was partially purified by precipitation with streptomycin sulfate and ammonium sulfate, gel filtration, and column chromatography; most of the biologic activity was precipitated by 40 to 70 percent ammonium sulfate. The precipitate was dissolved in a little distilled water, dialyzed, and run through a Sephadex G-100 column. At least three protein peaks were separated, most of the biologic activity being associated with the third peak. The active fractions were pooled, concentrated, and refractionated through a DEAE Sephadex A-50 column at *p*H 7.2; parabolic-gradient salt elution was used. Most of the biologic activity was localized in the fraction eluted with 0.1 to 0.2*M* sodium chloride (Fig. 1). When assayed with casein as substrate, the fraction revealed proteolytic activity. Biologic activity was tested in vitro on tissues dissected from chick embryos. Tissue fragments of about 1 mm^3 were explanted in Eagle basal medium in small falcon dishes and placed in an incubator conditioned with CO_2 at 38°C; they were then fixed and examined histologically.

Growth stimulation by the salivary-gland fraction was clearly evident in explants of lung from 8- to 9-day chick embryos. At this stage the lung primordia consist of loose mesenchyme in which bronchial tubules grow by a process of budding and branching; control cultures examined after 24 hours in vitro show fairly good maintenance of the original structure. Addition of the salivary-gland fraction at concentrations ranging between 5 and 50 μg per milliliter of culture medium caused marked changes: the mesenchyme underwent very active and vigorous growth, without appreciable change in the epithelium lining the bronchial tubules.

The growth-stimulating effect of the extract was further investigated by separating the two lung components: mesenchyme and bronchi. The bronchi were stripped from the surrounding mesenchyme, and mesenchymal and epithelial components were cultured in the presence of the active fraction. Mesenchymal tissue in control media underwent little change during 24 to 48 hours in vitro; there were some necrotic areas in the central part of the explants but the peripheral parts closely resembled the tissue of origin in cell density and in the loose texture of the intercellular matrix. Fragments in experimental cultures differed strikingly from the tissue of origin and from the cultured controls; a dense cell population replaced the loose—even scattered—fibroblasts of the original tissue, and the cells were arranged in tightly packed ribbons of spindle-shaped fibroblasts which filled all available space in the explants. As a result, there was a dense and vigorously growing cell population with no intercellular matrix (Fig. 2, *a* and *b*). Autoradiographic experiments with H^3-thymidine yielded evidence of many mitotic figures in the experimental cultures; such figures were practically absent in controls (Fig. 3, *a* and *b*).

Explants consisting of one or two bronchi stripped from the surrounding mesenchyme underwent little change when cultured for 24 to 48 hours in Eagle medium alone. In the presence of the salivary-gland fraction the few mesenchymal cells that still adhered to

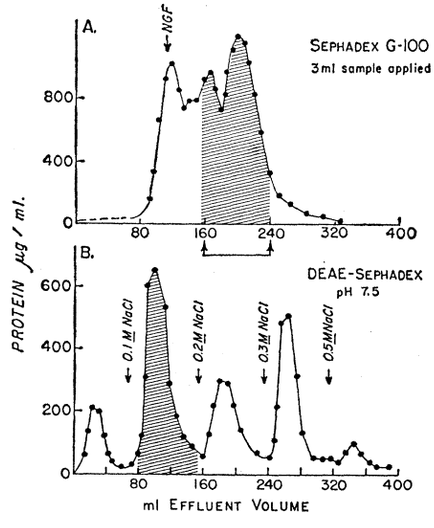

Fig. 1. (*A*) Fractionation of salivary-gland extract through Sephadex G-100 column after ammonium sulfate precipitation: activity of nerve growth factor localized in the first peak; biologic activity in the hatched area. (*B*) Further chromatography of the active fractions from Sephadex G-100 through a DEAE Sephadex column: stepwise elution with increasing concentration of sodium chloride; biologic activity in the hatched area.