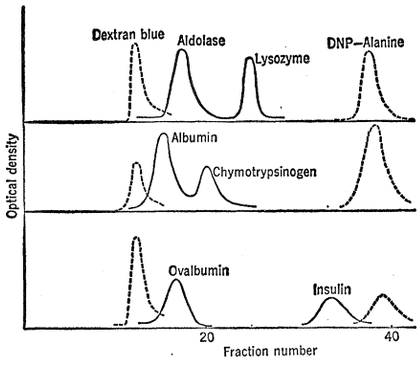

Fig. 1. Elution diagrams of proteins chromatographed on 6 percent agarose in 5*M* guanidine hydrochloride (top two curves) or 8*M* urea. Each solvent also contained mercaptoethanol and EDTA (see text). The profile of dextran blue was measured at 6300 Å; that of dinitrophenyl- (DNP) alanine, at 3500 Å. The turbidity of the protein solutions was measured at 4500 Å on a Gilford spectrophotometer.

the protein studied, V_o is the exclusion volume indicated by dextran blue, and V_i is the internal volume indicated by dinitrophenyl-alanine. For lysozyme in eight experiments K equaled 0.489 ± 0.016; for bovine serum albumin in five experiments, 0.100 ± 0.012.

Figure 2 shows the relation of the distribution coefficients to the molecular weights of the polypeptide chains studied by me. These results may be summarized by the expression $\log M = 4.01 - 2.105\,K$, where M is the molecular weight of the peptide chain. The

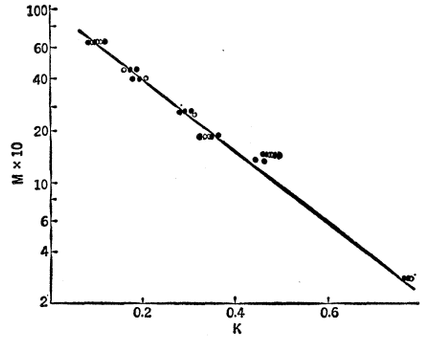

Fig. 2. Semilogarithmic plot relating the distribution coefficient K to the molecular weights of the polypeptide chains. The A and B chains of insulin were not resolved, and the logarithmic mean of their weights is used in the graph. Values for K were determined in guanidine hydrochloride (●) and in urea (○). The molecular weights of the chains were assigned as follows (in ascending order): insulin, 2,800; ribonuclease, 13,700; lysozyme, 14,500; β-lactoglobulin, 18,500; trypsin, 24,000; chymotrypsinogen, 25,000; aldolase, 40,000; ovalbumin, 45,000; and bovine serum albumin, 65,000. Lysozyme was consistently retarded from its expected position.

precision of these measurements suggests that the molecular weights may be estimated probably within 10 percent. The relation between K and M can certainly be expected to vary with different batches of gel, but calibration can be readily effected by chromatography of albumin, chymotrypsinogen, and insulin.

Several previous molecular-sieve studies of denatured proteins have been made without reduction of disulfide bonds (*4*). The intrinsic viscosities of peptide chains, denatured with and without breakage of disulfide bonds, are very different (*2*), and the importance of adequate reduction therefore merits emphasis if full denaturation is to be attained; that is, the concentration of thiol reagents must be sufficient to overcome any aerial oxidation that may occur, and a *p*H higher than 6 must be maintained to permit rapid reduction of disulfide (*2*). Another recommended addition to the denaturing solution is a chelating reagent to scavenge the metal from any metalloprotein so as to permit full unfolding of the polypeptide chains.

These experiments have demonstrated the utility of solutions of urea and guanidine hydrochloride, and concentrated solutions of lithium chloride probably could be used similarly. However, in view of the varying degrees to which detergent molecules are bound to proteins, the homologous behavior of detergent-denatured proteins cannot be assumed. The urea-treated proteins could suffer carbamylation during study; thus electrophoretic heterogeneity may be produced by the chromatography (*5*).

The validity of the molecular weights, determined by molecular sieving, must depend on the complete denaturation of the polypeptide chains and the absence of adsorptive interaction between protein and gel; for either of these reasons the molecular weight could be underestimated. The consistent retardation of lysozyme, in both urea and guanidine hydrochloride, presumably must be explained by one of these factors. Thus it is clearly desirable to confirm by some independent procedure, such as viscometry (*2*) or ultracentrifugation (*3*), any molecular weights determined by sieve chromatography. Conversely the molecular weights determined by these other procedures may well merit vindication by analysis by sieve chromatography, especially if the homogeneity of the polypeptide is in doubt.

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References and Notes

1. P. Andrews, *Lab. Pract.* **16**, 851 (1967).
2. C. Tanford, K. Kawahara, S. Lapanje, *J. Amer. Chem. Soc.* **89**, 729 (1967).
3. A. Ullmann, M. E. Goldberg, D. Perrin, J. Monod, *Biochemistry* **7**, 261 (1968).
4. E. C. Gotschlich and G. M. Edelman, *Proc. Nat. Acad. Sci. U.S.* **54**, 558 (1965); T. I. Přistonpil, *J. Chromatogr.* **19**, 64 (1963); T. Wieland, P. Duesberg, H. Determann, *Biochem. Z.* **337**, 303 (1963).
5. G. R. Stark and D. G. Smyth, *J. Biol. Chem.* **238**, 214 (1963).
6. Supported by NIH grant NB-00024.

28 June 1968

Uroporphyrinogen III Cosynthetase in Bovine Erythropoietic Porphyria

Abstract. *The activity of uroporphyrinogen III cosynthetase is much lower in hemolyzates from mature cattle with congenital erythropoietic porphyria than in hemolyzates from mature normal cattle. The porphyric hemolyzates do not inhibit the cosynthetase activity present in normal hemolyzates or in extracts of mouse spleen.*

Uroporphyrinogen III cosynthetase determines the structure of the tetrapyrrole formed by the enzyme uroporphyrinogen I synthetase (*1, 2*). In the presence of cosynthetase, synthetase catalyzes the conversion of the monopyrrole porphobilinogen to uroporphyrinogen III, a physiological intermediate in the biosynthesis of protoporphyrin IX. In the absence of cosynthetase, synthetase catalyzes the formation of uroporphyrinogen I, a product which is not converted to heme or chlorophyll by biological systems. The mode of action of cosynthetase is unknown; it does not catalyze interconversion of the uroporphyrinogen isomers. Bogorad proposed that synthetase catalyzes the formation of a linear polypyrrole which cyclizes spontaneously to form uroporphyrinogen I, and that cosynthetase is an enzyme which catalyzes cyclization of the polypyrrole to form uroporphyrinogen III (*3*).

Animals and humans with the hereditary disorder congenital erythropoietic porphyria excrete in the urine large amounts of uroporphyrin I, an oxidation product of uroporphyrinogen I. Increased excretion of uroporphyrin I could result from a hereditary deficiency of the cosynthetase (*3, 4*), but direct measurements of cosynthetase activities have not been made. Cosynthetase activity in crude extracts may now be measured with the use of partially purified synthetase from hematopoietic mouse spleen (*2, 5*). With this method,

the amounts of cosynthetase in hemolyzates from four mature porphyric cattle have been found to be much lower than the amounts in hemolyzates from four mature normal animals.

The assay for uroporphyrinogen III cosynthetase in crude tissue extracts depends on proportionality between the percentage of isomer III in the reaction product and the amount of the cosynthetase preparation added, under conditions where the total amount of uroporphyrinogen (I + III) is kept constant (*2*). Heparinized blood, drawn at the same time from a porphyric animal and a normal control, was the source of the cosynthetase used for these experiments. The specimens were frozen in solid CO_2 and maintained at −20°C until assayed. The paired porphyric and normal samples were assayed simultaneously, 2 to 5 days after the blood was drawn. Thawed hemolyzates were diluted with three volumes of 0.05*M* potassium phosphate buffer, *p*H 7.65. Portions of this dilution were incubated for 30 minutes at 31°C with 50 μmole of potassium phosphate buffer, *p*H 7.65, 0.12 μmole of tritium-labeled porphobilinogen, and enough uroporphyrinogen I synthetase, prepared from mouse spleen (*2*), to catalyze the formation of 5 mμmole of uroporphyrinogen (I + III), in a volume of 0.5 ml. The reaction was stopped with 0.035 ml of acetic acid. The uroporphyrinogen formed was oxidized to the porphyrin with 0.01 ml of 0.2*M* iodine in 0.3*M* KI, isolated on talc, esterified with methanolic H_2SO_4, and extracted into chloroform (*1*). The methyl esters were separated chromatographically (*6*), eluted from the paper with toluene, and counted in a liquid-scintillation system. Cosynthetase activity is expressed as the percentage of isomer III in the total ester recovered.

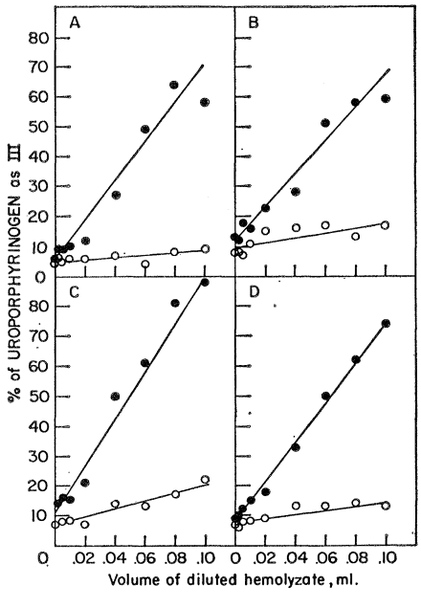

Fig. 1. Measurement of uroporphyrinogen III cosynthetase activity in diluted hemolyzates from normal and porphyric cattle. Closed circles, normal animals; open circles, porphyric animals. Hemoglobin determinations on the porphyric animals gave: 7.8, 11.9, 12.9, and 13.0 g per 100 ml of blood for porphyric animals A, B, C, and D, respectively; and 9.9, 9.1, 16.0, and 10.8 g per 100 ml of blood for the corresponding controls. The porphyric animals of pairs B and D and the normal animal of pair C were mature bulls; the others were mature cows.

The results obtained in the four paired assays are shown in Fig. 1. The assays on all the normal animals gave a linear relationship, rising as high as 89 percent of the product as isomer III with 0.1 ml of diluted hemolyzate (control animal C). In each case, the hemolyzate from the porphyric animal had much less effect than did the hemolyzate from the normal animal on the isomer composition of the uroporphyrinogen formed by synthetase from mouse spleen. In porphyric animals A and D, the cosynthetase activities of the amounts of hemolyzate tested were not significantly greater than the blank without added hemolyzate. The increment in percentage of III produced by 0.1 ml of diluted hemolyzate was from four to ten times greater in the normal than in the porphyric animals.

The hemolyzates from the porphyric animals did not interfere with the cosynthetase activity of hemolyzates from the normal animals. For example, in one experiment with 0.04 ml of the diluted hemolyzate from the normal animal D, the product contained 36 percent of isomer III; with 0.06 ml from the porphyric animal D, it contained 12 percent of III; and with the two combined, 39 percent of III. The product in the blank without hemolyzate contained 8 percent of III. Also, the hemolyzate from this porphyric animal did not inhibit the activity of cosynthetase prepared from mouse spleen (*2*).

Control experiments demonstrated that the amounts of hemolyzate assayed for cosynthetase did not have detectable synthetase activity under the reaction conditions, and did not stimulate or inhibit the mouse spleen synthetase. The amount of endogenous uroporphyrinogen which could be recovered as the methyl ester from the volumes of hemolyzate used for assay were less than 2 percent of the amounts formed in the reaction mixture.

The low concentrations of measurable cosynthetase in the hemolyzates of porphyric animals are thus probably due to a true lack of the cosynthetase activity, rather than to the presence of an inhibitor.

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References and Notes

1. L. Bogorad, in *Methods in Enzymology*, S. P. Colowick and N. O. Kaplan, Eds. (Academic Press, New York, 1962), vol. 5, p. 885.
2. E. Y. Levin and D. L. Coleman, *J. Biol. Chem.* **242**, 4248 (1967).
3. L. Bogorad, *Ann. N.Y. Acad. Sci.* **104**, 676 (1963).
4. S. Granick, *Trans. N.Y. Acad. Sci. Ser. II* **25**, 53 (1962).
5. E. Y. Levin, *Biochemistry*, in press.
6. J. E. Falk and A. Benson, *Biochem. J.* **55**, 101 (1953); P. A. D. Cornford and A. Benson, *J. Chromatogr.* **10**, 141 (1963).
7. I thank Dr. J. E. Smith, Dr. W. Medway, and J. Nordstrom for the hemolyzates. Supported by grant NB-05367 from NIH.

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Choline Acetyltransferase: Regional Distribution in the Abdominal Ganglion of Aplysia

Abstract. *Using a new microassay, we have determined the properties and the regional distribution of choline acetyltransferase in the abdominal ganglion of* Aplysia. *Enzyme concentrations in homogenates of groups of cells and in single identified cells indicate that neurons which function as neurosecretory cells, and which do not form chemical synapses with other cells or with peripheral structures, have little or no ability to synthesize acetylcholine; neurons which are involved in visceromotor integrations, and which connect with each other or with the periphery, have a substantial concentration of the enzyme.*

The abdominal ganglion of the marine mollusk *Aplysia californica* is particularly useful for the biochemical analysis of individual cells. Most of the neurons in this ganglion are large, some reaching 1 mm in diameter, and many are located near the ganglion's surface. The cells are readily seen under the dissecting microscope, and intact cell bodies without glial contamination can be isolated by manual dissection. Moreover, 30 of the 1000 to 2000 neurons in the ganglion can be iden-