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Immunological Approach -- Antibodies to a highly acid, brain-specific protein found in vertebrates, the S-100 protein, were obtained with the purified bovine S-100 complexed with methylated bovine serum albumin as immunogen. The presence of antigenic activity in brain extracts from a number of vertebrates measured by a quantitative complement (C') fixation technique indicated an unusually close serological relationship among these S-100 proteins.

When the purified bovine S-100 protein was heated in a tris-saline buffer (pH 7.4) for 10 min at temperatures from 20-60°, the per cent C' fixation, measured at 2-4°, gradually decreased and the antigen concentration giving maximum C' fixation increased. After heating at 70° or higher, no antigenic activity was detected. In the presence of 1×10^{-3} M EDTA, 2-mercaptoethanol or $CaCl_2$, the protein was protected from thermal denaturation. Divalent cations other than Ca^{++} did not exhibit this protective effect.

Studies of the optical rotatory dispersion (ORD) of bovine S-100 solutions in tris-saline buffer (pH 7.4) at various temperatures indicated that the dispersion curve displays a trough with a minimum at a wavelength of 233 mμ and that this trough value becomes less negative as the temperature increases. This may be interpreted as the destruction of α helical structure as the temperature is increased. Upon cooling the heated solution, the $[m']_{233\ m\mu}$, the reduced mean residue rotation at 233 mμ, reverted to the original value only if EDTA was present, to a lesser extent in the presence of mercaptoethanol or $CaCl_2$, and only partly in tris-saline buffer.

When the protein was dissolved in distilled water, ORD measurements showed that at 20°, the ORD curve had a maximum [m'] of 29,500 at 198 mμ and a minimum of about -6,000 at 233 mμ. Among computer generated ORD curves representing varying proportions of α helix, β and random chain, the one with 40% α helix, 30% β, and 30% random structure most closely resembled bovine S-100 in distilled water at 20°. As the protein solution was heated the $[m']_{198\ m\mu}$ value decreased and the $[m']_{233\ m\mu}$ became less levorotatory, indicating destruction of the α helix. However, with the rise in temperature, a peak gradually appeared at about 190 mμ. This was interpreted as the formation of a new protein conformation at high temperature, perhaps the parallel β structure. Solutions that have been heated to intermediate temperatures and then cooled retain a small amount of the 190 mμ peak and partly regain the 198 mμ peak. After 90° incubation, the cooled protein retained none of the $[m/]_{190\ m\mu}$ peak, but reverted to about 25% α helix.

Enzymological Approach. Aldolase -- Our studies on the heterogeneity of the multiple forms of aldolase in chicken tissues have demonstrated that a third parental type (aldolase C) occurs in chicken tissues along with its hybrid forms. These studies showed that three independent

genes that lead to the synthesis of aldolase A, B, and C polypeptide chains are responsible for the multiple forms of aldolase found in chicken tissues. Studies on chicken embryos have shown that during the initial stages of development, the activity of the A and C-type aldolases are present, but the principal embryonic form is aldolase C. The activity of B-type aldolase appears in liver and kidney during the later stages of development.

Aspartate Aminotransferase -- The soluble aspartate aminotransferase from chicken heart has been purified. Both the halo-and apoenzymes have been crystallized. The properties of the chicken heart enzyme have been compared to those reported for the enzyme isolated from pig heart, rat liver, and ox heart.

Arginine Kinases -- Our work on arginine kinase has consisted of comparative studies of the enzyme in various arthropod species. We have purified arginine kinase from three crustacean species, the lobster, blue crab, and the hermit crab. In addition, two electrophoretically distinct forms have been purified from the muscle of the horseshoe crab, Limulus polyphemus. Similar isoenzymic forms were separated in extracts from several spider species. Insect arginine kinases have been studied with crude extracts and partly purified systems. The purified preparations have many properties in common. Their amino acid compositions are similar. Each contains five cysteine and no cystine, and all are inhibited by sulfhydryl reagents. Their molecular weights as measured in the analytical ultracentrifuge and by chromatography on Sephadex G-100 range between 35-40,000. After inactivation by exposure to 8 M urea, the lobster enzyme can be reactivated by dilution into buffer containing a thiol, such as β-mercaptoethanol. L-arginine and ATP also promote reactivation but ADP is less effective in this regard. Magnesium and other divalent metal ions inhibit substrate-promoted reactivation. Similar studies on reactivation after treatment with 8 M urea have been conducted with the other purified arthropod arginine kinases. The two arginine kinase isoenzymes isolated from Limulus muscle differ in their stability at high temperatures, but they are very similar in their physical and catalytic properties. The two forms are not interconverted by treatment with 8M urea and reactivation, and C' fixation experiments indicate definite structural differences between the two forms.

Sequence of Dogfish M_4 Lactate Dehydrogenase (LDH) -- The determination of the total amino acid sequence of dogfish M_4 LDH has progressed in the following way. All of the peptides in a tryptic digest of the enzyme have been resolved by a combination of gel filtration, paper electrophoresis at various pH values, and paper chromatography. The amino acid compositions of these peptides have been determined and, in some cases, the N-terminal amino acid and partial sequences of these peptides have been determined.

LDH from the tail muscle of the American lobster (Homarus Americanus) has been purified to homogeneity as determined by ultracentrifugal analysis and starch gel electrophoresis. The physical properties of the enzyme have been characterized and are in general, similar to LDH's from vertebrate and

other invertebrate sources. The catalytic parameters, K_m, turnover number, optimal pyruvate concentration, and substrate inhibition, for the reverse reaction (Py + DPNH $\longrightarrow$ lactate + DPN^+) have been determined and are similar to LDH's reported in the literature. However, the catalytic properties for the forward reaction (lactate + $DPN^+ \longrightarrow$ Py + DPNH) differ significantly from LDH's hitherto studied. At low concentrations, the product DPNH increases the rate of the reaction and at high concentrations inhibits it; the inhibition is competitive with respect to DPN^+. The product activation causes the rate vs substrate concentration plots to assume a sigmoidal shape. The activation of the enzyme by DPNH is believed to be caused by conformational changes in the protein brought about by the binding of DPNH, which enhances the affinity of the enzyme for DPN^+.

Pseudomonas Mutant Enzymes -- Several enzymes isolated from Pseudomonas testosteroni mutant strains have behaved very differently from the wild type. On electrophoresis, it was found that the proteins in the crude extract from the mutant strain showed absolute migration toward the anode, whereas the wild type proteins migrated both toward the anode and cathode. All the enzymes isolated from the mutant strain, such as succinic dehydrogenase, glutamate dehydrogenase, malate dehydrogenase, steroid dehydrogenase, isocitrate dehydrogenase, glucose 6-phosphate dehydrogenase, showed more acidic properties than the wild type did. Another strain that was observed while the mutant was being grown, showed exactly the same properties as the wild type did. We are trying to compare in greater detail the properties of the proteins from these three related strains and eventually to study the differences in their genetic codes by sequencing either their DNA or RNA.

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