ASSEMBLY PROCESSES IN OLIGOMERS CONTAINING STRUCTURALLY DISTINCT SUBUNITS

Respiratory Proteins as Models

Celia Bonaventura and Joseph Bonaventura, *Duke University Marine Laboratory, Beaufort, North Carolina 28516 U.S.A.*
Marius Brouwer, *University of Groningen, 9747 A. G. Groningen, The Netherlands.*

There are two major classes of oxygen carrying proteins: the hemoglobins and the hemocyanins. Tetrameric hemoglobin is an oxygen carrier that has long served as a model in the analysis of allostery in proteins. In assembly processes as well, the oxygen carrying proteins appear to be good model systems which illustrate the distinct roles played by structurally diverse subunits. Tetrameric human hemoglobin shows definite differences in assembly and tetrameric stability depending on alpha-beta, alpha-alpha, beta-beta, alpha-gamma, etc., interactions. The blue-colored hemocyanins are found in the hemolymph of many molluscs and arthropods. In these molecules, oxygen binds at dimeric copper centers. The reactivity toward oxygen is typically modulated by external factors such as pH and sodium chloride (1, 2). Because of their extremely large size and subunit diversity, the hemocyanins may be particularly useful as assembly models.

Mollusc hemocyanins are commonly composed of 20 subunits whose molecular weights are about 400,000. We have found that in *Murex* hemocyanin the assembly of the oligomer is dependent upon the interactions of at least two distinct types of subunits (3). The assembled molecules are cylinders ~350 Å in diameter and 390 Å long. Limited proteolysis of the subunits in the "caps" of the cylindrical molecules promotes an end-to-end association resulting in the formation of enormous protein strands (4).

In arthropods, 75,000 dalton hemocyanin subunits associate into hexamers, dodecamers, 24-mers and 48-mers. In the blood of the horseshoe crab, *Limulus polyphemus*, the hemocyanin is a composite of eight hexameric structures and has a molecular weight of ~3.3 $\times$ 10^6. This molecule is assembled from at least eight distinct subunit types. Specific subunits play distinct roles in the assembly process (5). Moreover, subunits which play specific roles in the assembly of *Limulus* hemocyanin can play the same roles in the assembly of hybrid hemocyanins involving subunits from other species.[1]

The degree of aggregation of hemocyanin oligomers is influenced by external factors such as oxygen, pH, ionic strength, and the concentration of divalent cations. These factors stabilize different allosteric states. These states have differing degrees of quaternary constraint. This gives rise to dramatic differences in the time courses of dissociation of the oligomers when divalent cations are removed. Stopped-flow light scattering is a useful tool for analysis of the stability of various aggregation states in high molecular weight oligomers. Thus, in various hemocyanin systems it is possible to measure the rates of partial dissociation or reassociation by mixing solutions of hemocyanin with buffers containing chelators or buffers containing known amounts of stabilizing agents. By way of this technique, we have been able to differentiate between the stability of various aggregation intermediates. Notably, both oxy- and deoxy-*Limulus* hemocyanin can exist in a 48-subunit state, but the oxygenated

[1] van Bruggen, E. F. J., M. Bijlholt, W. Schutter, T. Wichertjes, J. Bonaventura, C. Bonaventura, J. Lamy, M. LeCler, H. J. Schneider, J. Markl, and B. Linzen. 1980. Manuscript submitted for publication.

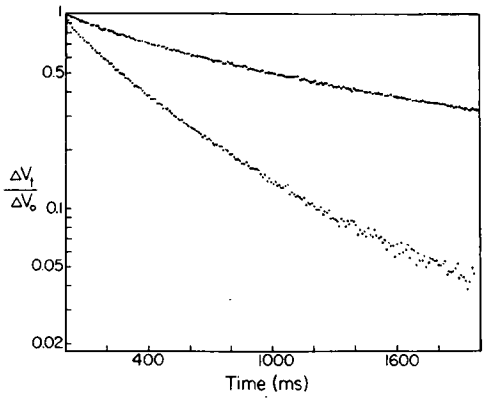

Figure 1 Stopped-flow light scattering experiments with oxy and deoxy forms of 60S *Limulus* hemocyanin. Oxy- or deoxy-hemocyanin in 50 mM Tris, ionic strength 0.05, pH 8.9, 10 mM $CaCl_2$ at 20°C and at a protein concentration of 5 mg/ml, was rapidly mixed with oxy- and deoxy-Tris buffer containing 50 mM EDTA, pH 8.9. Upper curve: deoxy hemocyanin mixed with deoxy-EDTA buffer. Lower curve: oxy-hemocyanin mixed with oxy-EDTA buffer and deoxy-hemocyanin with oxy-EDTA buffer. The dissociation into 5S particles was monitored by measuring the decrease of the light scattering intensity signal at 425 nm.

form of the 60S *Limulus* hemocyanin dissociates much more readily than does the deoxygenated form (Fig. 1). Sodium chloride, an allosteric effector of this system, stabilizes the contacts between subunits and greatly decreases the rate at which the 48-subunit molecule will dissociate into smaller fragments, even in the oxygenated condition (Fig. 2). The stopped-flow light scattering technique allows for analysis of many different experimental conditions much more readily than does ultracentrifugation, where only the final aggregation state is generally observed.

The fact that even large complexes assembled from diverse subunit types can show variation in structural stability in response to allosteric effectors suggests that the assembly of other large enzyme complexes and protein ensembles like ribosomes may likewise be under allosteric control.

Many enzyme systems that show allosteric behavior are composed of multiple types of subunits that coexist in an enzyme complex. Assembly processes in these heteropolymers are

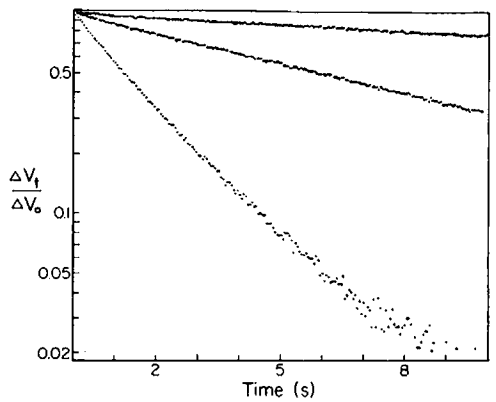

Figure 2 Experiments as in Fig. 1 with 60S oxy-hemocyanin and varied concentrations of NaCl in the protein solution. Upper curve = 0.3 M NaCl with hemocyanin; middle curve = 0.2 M NaCl; and lower curve = 0.1 M NaCl.

of interest in that the different subunits may participate in different ways in the assembly of the complex. The positioning of a subunit in the complex may be crucial to its function. The dynamic equilibrium between various assembly states of a heteropolymer cannot be treated as if the assembly involves structurally homogeneous elements whose association reactions can be described by a single interaction constant. On the contrary, each stage of assembly can be assumed to have a definite probability of adding another subunit which depends upon both the state of aggregation and the stereochemistry of the subunit. Problems of "micro-heterogeneity" in associating systems have puzzled, and plagued, researchers for decades. Observations of micro-heterogeneity in oligomers may to some extent be a consequence of nonequivalence of subunit types. An understanding of the intermolecular forces which govern the dissociation behavior of the mollusc and arthropod hemocyanins may provide an insight into the assembly of other proteins where functionally and structurally diverse subunits are known to play critical roles in the aggregation phenomena.

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ASSEMBLY OF CATALYTIC SUBUNITS OF ASPARTATE TRANSCARBAMOYLASE FROM *ESCHERICHIA COLI*

Drusilla L. Burns and H. K. Schachman, *Department of Molecular Biology and Virus Laboratory, University of California, Berkeley, California 94720 U.S.A.*

Although extensive studies have been conducted on the assembly of the allosteric enzyme, aspartate transcarbamoylase (ATCase) from isolated, intact catalytic (*C*) and regulatory (*R*) subunits, there has been little research on the formation of these subunits from individual catalytic (*c*) and regulatory (*r*) polypeptide chains. Such studies would be useful for evaluating the strengths of the interchain bonding domains within the subunits just as earlier experiments provided valuable data regarding interactions between the subunits in ATCase. The intact enzyme comprising two *C* trimers and three *R* dimers is designated as C_2R_3 or c_6r_6.

Isolated *C* trimers, in contrast to intact ATCase, exhibit Michaelian kinetics and no inhibition by CTP (or activation by ATP). The trimers are very stable and no dissociation has been observed in the ultracentrifuge even at concentrations <2 μg/ml. However, hybrids were detected when mixtures of native (C_N) and succinylated (C_S) subunits were incubated at 0°C for several days. The dissociation (and hybridization) of the subunits was decreased markedly (Fig. 1) upon the addition of the bisubstrate analog, *N*-(phosphonacetyl)-L-