Folding and association of oligomeric enzymes

R. Jaenicke

Institut für Biophysik und Physikalische Biochemie, Universität
Regensburg , Universitätsstraße 31 , D-8400 Regensburg , FRG

Nascent polypeptide chains fold spontaneously to their native three-dimensional structure, without the need for additional information beyond that contained in their specific amino acid sequence on one hand, and the aqueous or unpolar environment (for cytoplasmic or membrane proteins) on the other. The formation of oligomeric proteins implies association as an additional step. The native quaternary structure requires both folding and association to be properly coordinated [1].

In vitro reconstitution reflects the post-translational (or syn-translational) folding and assembly insofar as the final product of reconstitution after intermediary dissociation and unfolding is found to be indistinguishable from the native state [2]. In studying the correlation of folding and association of oligomeric enzymes the following problems will be considered:

1. Modes of dissociation/association
2. Mechanism and yield of reconstitution
3. Multimeric systems - multienzyme complexes.

1. Modes of dissociation/association. Dissociation, deactivation and denaturation have been shown to be inseparable processes, different modes of dissociation causing a differing extent of denaturation. As a consequence, reassociation is accompanied by more or less significant contributions of folding reactions to the overall kinetics of reconstitution. Under certain conditions (medium denaturant concentrations, specific "nicking" of enzyme molecules, high hydrostatic pressure etc), metastable intermediates of dissociation may be obtained [3-5]. These are found to be catalytically inactive.

To characterize the native and dissociated enzymes during the assembly process, "snap shot" techniques (e.g. hybridization or chemical cross-linking [6,7]) have to be applied. In certain cases, proton release, or spectral changes accompanying quaternary structure formation have been used as probes for kinetic analyses.

2. Mechanism and yield of reconstitution. The overall process of reconstitution represents a superposition of folding reactions with a sequence of bimolecular association steps:

$$nM \xrightarrow{uni} nM' \xrightarrow{bi} \frac{n}{2}D \xrightarrow{uni} \frac{n}{2}D' \xrightarrow{bi} \frac{n}{4}T \xrightarrow{uni} \frac{n}{4}T' \;\ldots$$

The outcome of systematic studies on a variety of oligomeric enzymes may be summarized as follows [1] :

(i) In the case of tetramers, inactive dimeric intermediates are formed in a fast (diffusion controlled) reaction, followed by the rate-determining tetramerization which is parallelled by reactivation. The respective second order rate constants of tetramer formation vary in a wide range.

(ii) Out of 11 different enzymes investigated so far, aldolase from rabbit muscle is the only example showing (partial) subunit activity; in all other cases catalytic activity requires the native quaternary structure.

(iii) Ligands may stabilize the nascent structure thus causing the yield of reconstitution to be increased; in certain cases effects on the kinetics have been approved [8,9].

(iv) Formation of non-covalent "wrong aggregates" competes with reconstitution; the profiles of both processes are complementary. As suggested by electron microscopy and CD, the aggregates consist of individual monomeric chains with partially restored secondary structure and $\underline{M}_r > 10^6$ [10]. Effects of incubation time and temperature clearly contradict proline cis-trans isomerization to be involved in the aggregation mechanism.

(v) As shown by joint reconstitution of closely related enzymes the assembly process is highly specific [11].

3. Multimeric systems - multienzyme complexes. As indicated by successful attempts to achieve reconstitution of complex biological structures, there are no qualitative differences to be expected in extending the scope of the foregoing studies towards multimeric systems. A new facet is the possible cooperativity or interdependence of different polypeptide chains in the joint folding and association of various subunits, forming the complex. Elementary processes of self-assembly were investigated using the coat protein of TMV as a model [12]. Reconstitution of the tryptophan synthase "bi-enzyme complex" seems to be governed by a uni-bi-unimolecular reaction with a rate-determining reshuffling process [7]. Pyruvate dehydrogenase from Bac. stearothermophilus ($\underline{M}_r \sim 10^7$) may be reactivated after acid dissociation, regenerating the native multienzyme complex. The overall reaction obeys first order kinetics and may be described by consecutive folding, association and reshuffling.

[1] Jaenicke, R. and Rudolph, R. (1980) in R. Jaenicke (Ed.) Protein Folding, Elsevier-North Holland, Amsterdam, pp.525.
[2] Jaenicke, R. and Rudolph, R. (1977) FEBS Symp. 49, 351.
[3] Jaenicke, R., Vogel, W. and Rudolph, R. (1981) Eur. J. Biochem. 114, 525.
[4] Girg, R., Rudolph, R. and Jaenicke, R. Eur.J.Biochem.in press
[5] Müller, K., Lüdemann, H.-D. and Jaenicke, R. (1981) Biophys. Chem. in press ; Biochemistry in press.
[6] Hermann, R., Rudolph, R. and Jaenicke, R. (1979) Nature 277, 243 ; Biochemistry in press.
[7] Seifert, T., Bartholmes, P. and Jaenicke, R., submitted.
[8] Jaenicke, R., Krebs, H., Rudolph, R. and Woenckhaus, C. (1980) Proc. Natl. Acad. Sci. USA. 77, 1966.
[9] Gerschitz, J., Rudolph, R. and Jaenicke, R. (1978) Eur. J. Biochem. 87, 591.
[10] Zettlmeißl, G., Rudolph, R. and Jaenicke, R. (1979) Biochemistry 18, 5567.
[11] Jaenicke, R., Rudolph, R. and Heider, I. (1981) Biochem. International 2, 23.
[12] Jaenicke, R. and Lauffer, M.A. (1969) Biochemistry 8, 3083.
Vogel, D. and Jaenicke, R. (1974) Eur. J. Biochem. 41, 607.
Lauffer, M.A. (1975) Entropy-Driven Processes in Biology, Springer, Berlin, Heidelberg, New York, 264 pp.