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The hemoglobin cyanomet ligation analogue and carbon monoxide induce similar allosteric mechanisms☆

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Abstract

Current thermodynamic models of protein cooperativity predicting sigmoidal ligand equilibrium curves differ in the assumptions regarding the structural/functional properties of the intermediate ligation states. Quantitative information on the intermediates cannot be extracted from the equilibrium curves, but must be obtained from direct studies of the intermediates. Since the intermediates are intrinsically unstable species, ligation analogues with reduced mobility are indispensable tools for cooperativity studies provided that the tertiary/quaternary changes triggered by the ligation analogue are similar to those observed using the physiological ligands. We demonstrate that the valency exchange reactions occurring in mixtures of deoxy and cyanomethemoglobin yield non-random distributions of deoxy/cyanomet intermediates that resemble those observed in the equilibrium with carbon monoxide. Previous and new data using the analogue, in agreement with the studies of the CO intermediates, indicate that the mechanism of hemoglobin cooperativity is neither purely concerted nor sequential nor combinatorial, but contains some elements of each of these models.
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1. Introduction

Allosteric proteins, either single polipeptide chains or assemblies of functional chains, play a key role in the regulation of fundamental biological processes. In general, the mechanisms by which they fulfill such a role exploit the flexibility of these molecules allowing them to take up different conformations upon binding specific ligands. In most cases of allosteric assemblies of protein functional units the ligand binds in a cooperative positive mode yielding equilibrium curves sigmoidal in shape [1]. Sigmoidal equilibrium curves are predicted by thermodynamic models of cooperativity, which differ in the assumptions regarding the structural/functional properties of the intermediates [2–4]. The nature and the concentrations of

Abbreviations: Hb, HbO_2, HbCO, HbCN, Hb^+, deoxy-, oxy-, carbon monoxy-, cyanomet- and methemoglobin, respectively; α^{CN} and β^{CN}, cyanide bound hemoglobin chains in the ferric state; O.P. optical path.

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$(\alpha^1\beta^1)(\alpha^2\beta^2)$

$(\alpha^L\beta)(\alpha\beta)$ $(\alpha\beta^L)(\alpha\beta)$

$(\alpha^L\beta^L)(\alpha\beta)$ $(\alpha^L\beta)(\alpha\beta^L)$ $(\alpha^L\beta)(\alpha^L\beta)$ $(\alpha\beta^L)(\alpha\beta^L)$

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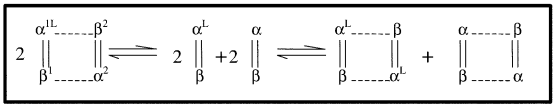

Fig. 1. The 10 hemoglobin ligation states. Superscript *L* indicates ligation. Superscripts 1 and 2 identify the four chains. The α^1–β^1, and α^2–β^2, contacts of the dimeric units, shown in parentheses, do not dissociate under physiological conditions. The weaker α^1–β^2 and α^2–β^1 inter-dimeric contacts allow a reversible tetramer–dimer dissociation. Symmetrical tetramers made up of dimers in the same ligation state are stable in pure form, since they re-associate into the original tetramer. Asymmetrical tetramers (insert) disproportionate into a ternary mixture of the asymmetrical and its parental symmetrical tetramers.

the molecules in a partial state of ligation, or intermediates, depend on the structure of the allosteric protein and the mechanisms of the cooperative interactions of the functional units. Therefore the determination of the properties of the intermediates is crucial for the choice of the model that is more adequate for the description of the cooperative mechanisms. Hemoglobin has a prototypic role in the study of cooperative proteins because of its physiological importance, the vast amount of data available on the structural/functional properties in the unliganded and fully liganded state, and, as an unique case among the cooperative proteins, because important thermodynamic properties of its intermediates are accessible to study. Ligand binding to hemoglobin yields the eight intermediates shown in Fig. 1. The study of the equilibrium curves cannot provide quantitative information on the type and properties of all these intermediates. Due to the dimer–tetramer equilibrium the intermediates that are accessible to study in a pure form are the symmetrical species, which dissociate into identical dimers. The asymmetrical intermediates can only be studied in mixture with the two symmetrical parental species, as illustrated in the insert of Fig. 1. The study of the intermediates in the reactions with gaseous heme ligands, such as O_2 and CO, is difficult because of the high mobility of the ligands, which dissociate from and re-associate to the four chain hemes. Nevertheless, the reduced CO mobility, as compared with that of O_2, has made it possible to trap and identify the CO intermediates under equilibrium and dynamic conditions using cryogenic analytical techniques [5,6]. The analyses of the equilibrium distributions have provided information on the thermodynamic properties of some CO intermediates [7,8]. To directly study the intermediates, either pure or in ternary mixtures, the ligand should have a low mobility compared with the time scale of the methodologies used for the study. To this end two types of ligation analogues have been used: non-native metal substituted hemoglobins, in which metals replacing the heme ferrous iron mimic the state of ligation or non-ligation, and cyanomet analogues, in which cyanide bound to ferric chains mimics the ligation state [9]. The cyanomet analogues, inexpensive and simple to prepare, have been intensively studied because of the assumed lack of mobility in comparison with the gaseous ligands. This assumption was incorrect, since these analogues undergo slow valency exchange reactions by which unliganded ferrous chains exchange electrons or the whole heme group with the cyanoferric chains [10]. The techniques that detect the valency exchange indicate that these reactions require several days to reach an apparent equilibrium, but do not provide information on the nature and distribution of the products of the exchange [10,11]. Such information is crucial for an assessment of the validity of the cyanomet analogue to represent cooperativity in ligation. Since the valency exchange reactions are slow, kinetic and thermodynamic properties of some intermediates can be obtained under condition of non-equilibrium, when the contamination by the products of the exchange is modest. However, if the valency exchange reactions yielded at equilibrium random distributions of intermediates,

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