Stereochemical Interpretation of High Oxygen Affinity of Haemoglobin Little Rock ($\alpha_2\beta_2{}^{143\text{His}\rightarrow\text{Gln}}$)

BROMBERG *et al.* have reported interesting observations on a new abnormal haemoglobin in which histidine H21(143)β is replaced by glutamine[1]. In normal deoxyhaemoglobin, the side chain of this histidine binds 2,3-diphosphoglycerate (DPG), and in normal oxyhaemoglobin it is free[2–4]. In the absence of DPG our electron density maps show it to be free and surrounded merely by water in both the normal deoxy and oxy forms[5,6], which implies that this side chain can have no measurable effect on the oxygen affinity; yet Bromberg *et al.* find the oxygen affinity of haemoglobin Little Rock to be high even in the absence of DPG.

I have examined the stereochemical effects that may arise through the amino-acid replacement in this haemoglobin variant. The atomic models of haemoglobin show that residue 143β lies in the internal cavity near the two-fold symmetry axis. In the oxy structure, the abnormal glutamine side chains would form hydrogen bonds with the side chains of asparagine H17(139) of the opposite β chain. On transition to the deoxy structure, the two β chains move apart so that these hydrogen bonds would be broken.

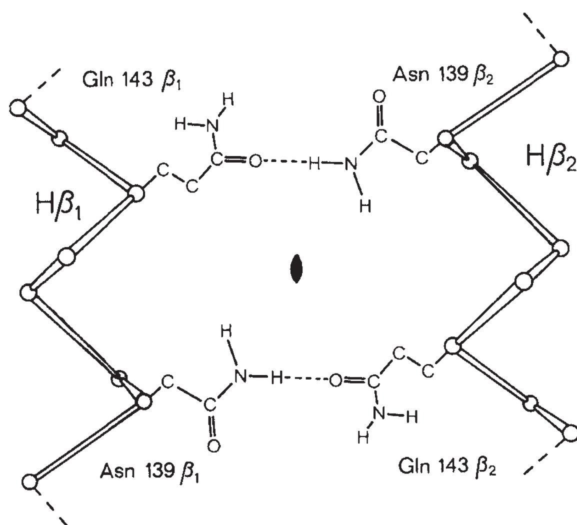

Fig. 1 Proposed hydrogen bonding between the two H helices in oxyhaemoglobin Little Rock. The normal histidine side chains cannot form these bonds.

Changed oxygen affinities often arise through shifts in the allosteric equilibrium between the two alternative structures of haemoglobin[7]. In haemoglobin Little Rock there would be two effects that raise the oxygen affinity; the oxy structure is stabilized by two additional hydrogen bonds between the β chains, and the deoxy structure is destabilized by the loss of two salt bridges to DPG. If my interpretation is correct, then carbonmonoxy and oxyhaemoglobin Little Rock should exhibit a diminished dissociation constant from tetramers to αβ dimers. Moreover, the raised oxygen affinity of "stripped" haemoglobin Little Rock should be chiefly a consequence of an increase in K_4, the association constant of the last oxygen to combine with the tetramer.

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Depletion of Synaptosomal Neurotransmitter Pool by Sudden Cooling

AN increasing number of reports deal with the uptake, storage and release of putative neurotransmitters by synaptosomes (pinched-off nerve endings)[1–6]. In most of these, incubated synaptosomes were rapidly cooled to 0°–4° C and washed, in order to measure the uptake or to study the release of the accumulated neurotransmitter[1,6,7–10]. Synaptosomes are usually collected by filtration through cellulose ester filters ('Millipore'), followed by washing with ice-cold medium[7–10]. We noted previously a large loss of accumulated and of endogenous GABA and glutamate during collection of synaptosomes on filters[11] and here we suggest that this loss is caused by an alteration of the membrane structure induced by sudden cooling and that the different depletion observed with various neurotransmitters may depend on their intrasynaptosomal compartmentation.

Crude synaptosomal preparations and purified synaptosomes[12] from adult rat cerebrum were incubated and collected as described in detail elsewhere[11,13]. The following labelled compounds were used: 2,3-^{3}H-γ-aminobutyric acid (GABA), 2 Ci mmol^{-1}; 2-^{3}H-glycine, 11 Ci mmol^{-1} and 1,2-^{14}C-taurine, 2 mCi mmol^{-1} (New England Nuclear Corp.); U-^{14}C-glutamic acid, 10 mCi mmol^{-1}; and L-noradrenaline(methylene-^{14}C)-D-bitartrate, 57 mCi mmol^{-1} (Amersham Radiochemical Centre).

At least 80% of ^{3}H-GABA and ^{14}C-glutamate was recovered unchanged in the tissue, as tested by column chromatography[11]. In similar experimental conditions, tritiated water excluded, more than 80% of the other compounds was not metabolized[3,4,6,14,15].

As the uptake of neurotransmitters by brain homogenates is chiefly due to synaptosomes[2,3] and as preliminary experiments showed crude and purified preparations of synaptosomes to behave similarly, crude preparations were generally used in this study.

When crude or purified synaptosomes, incubated with various putative neurotransmitter amino-acids, were collected on 'Millipore' filters and washed with ice-cold medium, the particles lost about 70% of the accumulated radioactivity. Washing with warm medium drastically reduced the loss (Table 1). Norepinephrine behaved differently: the loss was only 20% with cold medium and lower than 10% with medium at 37° C. The loss of GABA and glutamate was analysed in more detail. The results obtained with the two amino-acids were similar and Fig. 1 presents the data for GABA. With medium at 0° C, most of the loss of radioactivity occurred during the first 5 ml. washing. With medium at 37° C, the loss was much lower and more gradual. Qualitatively similar results were obtained using 0.32 M sucrose, although the recovery of radioactivity was somewhat greater with sucrose than with medium, at both temperatures. The retention of radioactivity by synaptosomes obviously depends chiefly on the temperature of the washing solution. Table 2 shows that a large loss of radioactivity occurs only when synaptosomes

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