CHAPTER 5
(Result and Discussion)
5.1 GLYCATED HEMOGLOBIN – PLATELET DEPENDENT NO TRANSFER

5.1.1 ODQ Inhibits the Deaggregatory Response of nitrosylated GHb

Fig. 5.1.1 describes the platelet deaggregation pattern of nitrosylated GHb (left panel). The 2nd, 3rd, 4th and 5th figure shows the platelet effect in presence of GHb and ODQ at different concentrations. And the right panel shows the effect of agonist ADP only.
The platelet aggregation profile in Fig.5.1.1 was studied in presence of the agonist adenosine diphosphate (ADP) and GHb. The inhibition of the agonist-induced aggregation by GHb was imminent. The ADP induced aggregation exhibited reverse curvature implying the interplay of a deaggregating response. As platelet aggregation has been used as an important bioassay for NO, so whether this anti-aggregatory response is the effect of NO or not was investigated by using ODQ (inhibitor of NO mediated platelet function). The extreme left panel of Fig.1 shows the platelet aggregation profile in presence of nitrosylated GHb. And the consecutive figures show the effect of ODQ added in various concentrations (0.5μM - 2μM) in presence of GHb. ODQ shows a graded platelet pro-aggregatory effect with increase in concentration. The pro-aggregatory platelet response of ADP as a control is also illustrated in the extreme right panel.
5.1.2 The Dilution Effect of nitrosylated Hb and nitrosylated GHb

Fig. 5.1.2 is the effect of nitrosylated Hb (left panel) and nitrosylated GHb (right panel) on successive dilution.

The comparative study of NO transfer by nitrosylated HbA0 and GHb is shown in Fig. 5.1.2. Nitrosylated Hb (left panel) and GHb (right panel) cause differential aggregatory responses. The colors (RGB) represent successively diluted protein solutions (0.64ug/ml, 0.426ug/ml and 0.320ug/ml). It may noted that GHb causes a conspicuous de-aggregatory response (marked by the tail portion of the aggregation profile). Such responses from the nitrosylated form of protein can be accounted for by considering that NO is transferred from the protein to the platelet.
5.1.3 The Platelet Profile of Hb in Different Ligand Form

Fig. 5.1.3 shows the platelet behaviour of oxyHb (upper left panel), deoxyHb (upper right panel), ferricnitrosoHb (lower left panel) and ferrousnitrosoHb (lower right panel) respectively.

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Fig. 5.1.3 is the platelet pattern in presence of the agonist ADP and Hb in different liganded form. The upper left panel and the upper right panel shows the effect of oxy Hb and deoxy Hb (prepared by addition of dithionite to oxy Hb) on platelet respectively. The Hb either in oxy or in deoxy state has the pro-aggregatory effect. The reagents in the lower two panels are ferricnitroso Hb (soret peak at 417nm) and ferrousnitroso Hb (soret peak at 419nm). Both the forms of Hb in these two liganded state again shows anti platelet aggregation. the ODQ mediated response was same as in Fig. 5.1.2. The figure depicts the platelet behaviour in different heme (redox) state.
5.1.4 The Differential Effect of Glucated and Fructated Hb

Fig. 5.1.4 The platelet aggregation profiles in presence of Hb (blue), GHb (red) and FHb (green), the samples being prepared by invitro method.
Fig. 5.1.4 summarises the platelet profile in presence of Hb as a control, GHb (Glycated Hb) and FHb (Fructosylated Hb) prepared by invitro method using ADP as agonist. The figure clearly illustrates that GHb (red) induces hyperaggregability in platelets as compared to HbA0 (blue). The pathological disorder that may follow from such hyperaggregibility is evident. The other notable result is the differential effect induced by fructated Hb (green) that causes mild loss of aggregation.

5.1.1 – 5.1.4 The Hb and glycated Hb shows differential platelet response either nitrosylated or not. The fructosylated Hb on the other hand has much different effect on platelet aggregation pattern from Hb or GHb.
5.2 DISCUSSIONS

Inhibition of platelet aggregation and the vasodilatory effect are the two major physiological functions attributed to NO. As well known in literature (see Fig. 1.2 in section 1.10) hemoglobin molecules serve as a NO sink, and to be more precise a reversible NO sink that also serves as a NO store. In this chapter we have studied how the store like and sink like activities of hemoglobin is affected when it undergoes glycation. The fact that glycation causes elevation of aggregation of platelets can be explained by the fact that glycation is associated with a partial unfolding of the protein. Such unfolding would expose the cysteine groups of the protein more effectively, and this will help transfer of NO from platelet to protein by means of S nitrosylation. It may be seen that fructation, that leads to AGE formation does not cause any hyperaggregation of platelets. The mechanism of the AGE mediated inhibition of platelet response deserves further studies.

This hyperaggregibility of platelets is eventually a common observation among diabetes patients, and it is this that makes the diabetic population more prone to thrombotic attack (77). But the reverse case, namely NO transfer from protein to platelets also deserves further attention. The faster transfer of NO from nitrosylated protein to platelets may be mediated by the same route, namely exposed cysteine groups. The other possibility namely, altered heme orientation can be ruled out as we have seen that the platelet response is partially independent of heme orientation. Higher de-aggregability shown by glycated proteins, though a bit paradoxical, is basically other side of the same "coin", namely partially unfolding induced by the glycation process.

Lastly, the results may throw some new lights in understanding the systems regulations involved in diabetes. The fact that NO is a signalling molecule, and fact that both NO absorption and release are favored by the glycated protein suggests that the glycation process would interfere with the NO signaling, and such enhanced NO transfer may partially compensate for the stress related events (NO being a relaxing molecule) associated with diabetic patients.