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PROTEIN-PROTEIN INTERACTIONS:POSSIBLE LOCATION OF HEMOGLOBIN-HAPTOGLOBIN CONTACTS

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SUMMARY : We have studied the binding of α and β chain tryptic peptides to Hp linked to agarose. The results obtained provide evidence for a specific binding of peptides to Hp. Addition of Hb, which binds irreversibly Hp resulted in a specific displacement of the peptides bound to Hp. The analysis of the peptides displaced and their location in the Hb molecule suggested that Hb interacts with Hp through surfaces by which (I) αβ dimers and (II) α and β monomers face each other in the Hb tetramer.

The reaction between haptoglobin (Hp) and hemoglobin (Hb) has a one to one stoichiometry and is effectively irreversible (1); isolated α chains bind Hp reversibly, while the affinity of β chains is very low (2, 3). Although the Hp-Hb reaction and the resulting complex have been extensively studied (4, 5), the precise location of Hp/Hb contacts remains indeterminate. On the basis of kinetic studies of the Hp-Hb reaction, it has been suggested that the binding of Hb to Hp occurs through αβ dimers (2) and an intermediate complex, in which the αβ dimer is bound to Hp (6). Although the deoxy Hb tetramer does not bind Hp, it has been demonstrated recently (7) that it dissociates, with a half-time of the order of several hours, into dimers, which then bind Hp. The dissociation of Hb into αβ dimers is a prerequisite for its binding to Hp.

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In the present report, the binding of the tryptic peptides of the α and β chains to Hp was examined with the aid of a haptoglobin-agarose affinity adsorbent (Aga-Hp), which was found to maintain the properties of Hp in solution (8). It use allowed a rapid separation of non-bound from ligands bound to Hp molecules. The specific displacement of Hb peptides bound to Aga-Hp was studied, and analysis of the eluted peptides has provided information on the areas of α and β chains which participate in the Hp/Hb contacts.

MATERIALS AND METHODS

Human Hp_{2-1} was purified from pleural fluids of patients with malignant diseases (9). Coupling of Hp to agarose (Sepharose 4B) was effected by cyanogen bromide activation of agarose (10). Hemolysates were prepared from fresh red cells (11). Apohemoglobin was obtained by acid-acetone precipitation at -20°C (12) and then separated into α and β globin chains by carboxymethylcellulose column chromatography in 8M urea buffers (13). Globin chains were aminoethylated (14) and subsequently digested with trypsin (15). Analytical fingerprints of the tryptic peptides were made (16) on thin layer silica gel plates (SCHLEISCHER and SCHULL). Peptides were stained with ninhydrin and by specific staining (17).

^{14}C-labelled Hb was obtained by incubation of normal human reticulocytes with L - [^{14}C] - leucine and L - [^{14}C] - lysine (18). ^{14}C-labelled α and β chains were made similar in specific activity (100 mCi/mol) by mixing cold and labelled material. All the tryptic peptides were ^{14}C labelled, except αT_{14} (140-141) and βT_{15} (145-146), which contain neither lysine nor leucine residues.

Experiments of peptide binding to Aga-Hp were carried out in CH_3 $COONH_4$ (5×10^{-2} M) buffer at pH 5.0. Peptides were added to Aga-Hp and stirred gently overnight at 4°C ; non-bound peptides were subsequently washed out with the same buffer. In experiments in which deoxy Hb was used, the buffer was previously degassed and made 10^{-2} M in sodium dithionite. The amount of Hb (or deoxy Hb) added to displace the bound peptides was saturating for Hp. Human IgG (Cohn fraction II) was added in the same molar amounts as for Hb.

Experiments on the inhitibion of α chain binding to Hp were carried out using ^{14}C-labelled α-PMB chains (600 mCi/mol), which were obtained after treatment of Hb by p-chloro-mercuri-(^{14}C)-benzoic acid (873 mCi/mol) (19). The ^{14}C radioactivity was counted in a Beckman LS-150 liquid scintillation system, after peroxidation of the samples, with adequate quenching corrections.

The tryptic peptides of the κ light immunoglobulin chain were prepared as previously described (20).

RESULTS AND DISCUSSION

Studies on the staphylococcal nuclease peptides (21) demonstrated that they are in conformational equilibrium in solution between native and random conformations (the term random is used to describe all the possible non-native conformations). These observations indicate that oligopeptides can bind to the corresponding site when they are in their native conformation. Indeed Fig. 1 shows that the binding of ^{14}C-labelled α-PMB chains to Aga-Hp is inhibited by addition of the soluble tryptic digest of α chains, while the addition of the tryptic digest of β chains and κ light immunoglobulin chains has little or no effect on this binding . These results gave evidence for competition between some α tryptic peptides and α chains for the binding sites of Hp.

When ^{14}C-labelled α chain peptides were incubated with agarose (Fig. 2I), addition of oxy Hb (Fig. 2IB) displaced radioactivity at the same level as addition of buffer (Fig. 2IA). Similarly when buffer (2IIA), IgG (2IIB) and deoxy Hb (2IIC) were added to Aga-Hp bound to α chain radioactive peptides, the amounts of radioactivity displaced are equal. In contrast addition of oxy Hb (2IID), which irreversibly binds Hp, displaced an amount of ^{14}C-labelled peptides, 3 times higher than that released under the preceding conditions.

The presence of a non-specific binding of peptides to agarose is evidenced by the radioactivity displaced when 6 M guanidine is added to agarose (Fig. 2IC).

The existence of a specific binding of α tryptic peptides to Aga-Hp was supported by the identical effect of 6 M guanidine whether it was added to agarose (2IC) or to Aga-Hp after addition of oxy Hb (Fig. 3IB). When 6 M guanidine was added prior to oxy Hb, there is no specific displacement of peptides (Fig. 3II). These results strongly suggest that the peptides released by addition of oxy Hb (2IID) occupied the binding sites of Hp and were displaced by Hb.

Aga-Hp (230 nmols of active Hp) was incubated with α or β chain ^{14}C-labelled peptides (9 x 10^5 cpm). Addition of oxy Hb

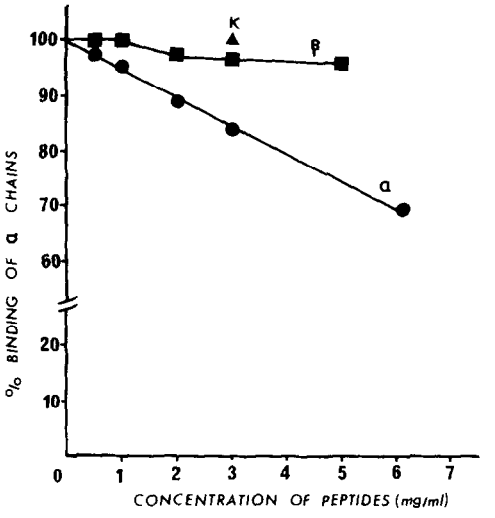

Figure 1. Binding of ^{14}C-labelled chains to haptoglobin in the presence of the tryptic digest of α aminoethylated (●——●), β aminoethylated (■——■) chains and κ carboxymethylated (▲) light immunoglobulin chains. One ml of sedimented Aga-Hp was mixed with 1 ml of peptide solution and stirred gently for 1 hour. ^{14}C-labelled α chains (0.5 ml) were then added. The final concentrations of α chain and Hp were 3.09 x 10^{-5} M and 6.11 x 10^{-6} M respectively. Measurement of non-bound α chains was performed 30 min. after their addition. Under the same conditions, in the absence of added peptides, the α chains saturated 25% of the binding sites of Hp. These experiments were carried out at 4°C in potassium phosphate (10^{-1} M) at pH 7.4 containing 10^{-3} M EDTA. Each sample was assayed in triplicate.

resulted in a displacement of 3101 cpm of α chain peptides and of 1359 cpm of β chain peptides.

The eluates were subsequently analysed by fingerprinting (Fig. 4) ; the eluate of tryptic peptides contained : αT_2 (8-11), αT_3 (12-16), αT_4 (17-31), αT_5 (32-40), αT_6 (41-56), αT_{8-9} (61-90) and αT_{10} (91-92) while that of the β tryptic peptides contained only βT_4 (31-40).

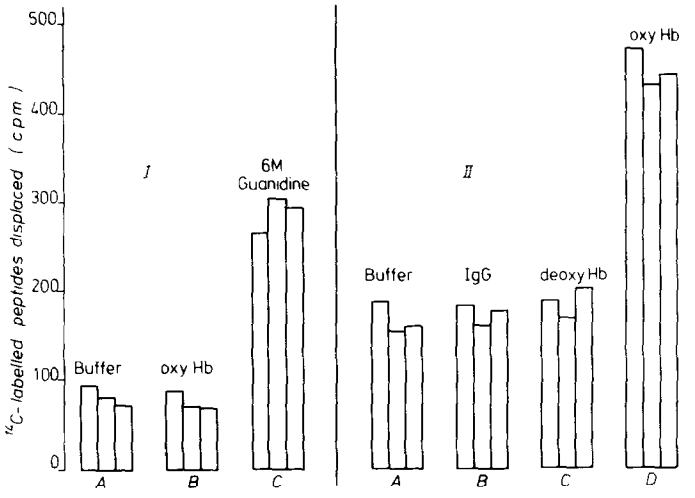

Figure 2. 15 ml of sedimented agarose (I) and Aga-Hp (II) were incubated with ^{14}C-labelled α chain peptides (10^5 cpm). The final volume was of 30 ml. After removal of non-bound peptides, 2 ml of buffer (IA), 25 μM oxy Hb (IB) and 6 M guanidine (IC) were added to samples (2 ml) of sedimented agarose, reacted with peptides. Addition of 2 ml of buffer (IIA), 25 μM IgG (IIB), 25 μM deoxy Hb (IIC) and 25 μM oxy Hb (IID) to samples of 2 ml sedimented Aga-Hp bound to peptides, was also effected. Each sample was assayed in triplicate.

It is noteworthy that the tryptic peptides displaced by Hb contain amino-acid residues of the globin moiety which participate in : a) globin-heme, b) $\alpha_1\beta_2$ and c) $\alpha_1\beta_1$ interactions. It is highly probable the Hp residues interact with residues of the globin moiety on the surface of the chains of Hb. The residues of Hb implicated in globin-heme interactions are buried in the interior of the molecule. Consequently they do not participate directly in Hb/Hp interactions.

Our results suggest that Hb interacts with Hp through surfaces by which (I) αβ dimers and (II) α and β monomers face each other in the Hb tetramer. The first point (I) is in agreement with the suggestions of other investigators (2, 6, 22). ORD studies of Hb-Hp complex (23) have suggested implication of $\alpha_1\beta_1$ contacts in Hp-Hb interactions.

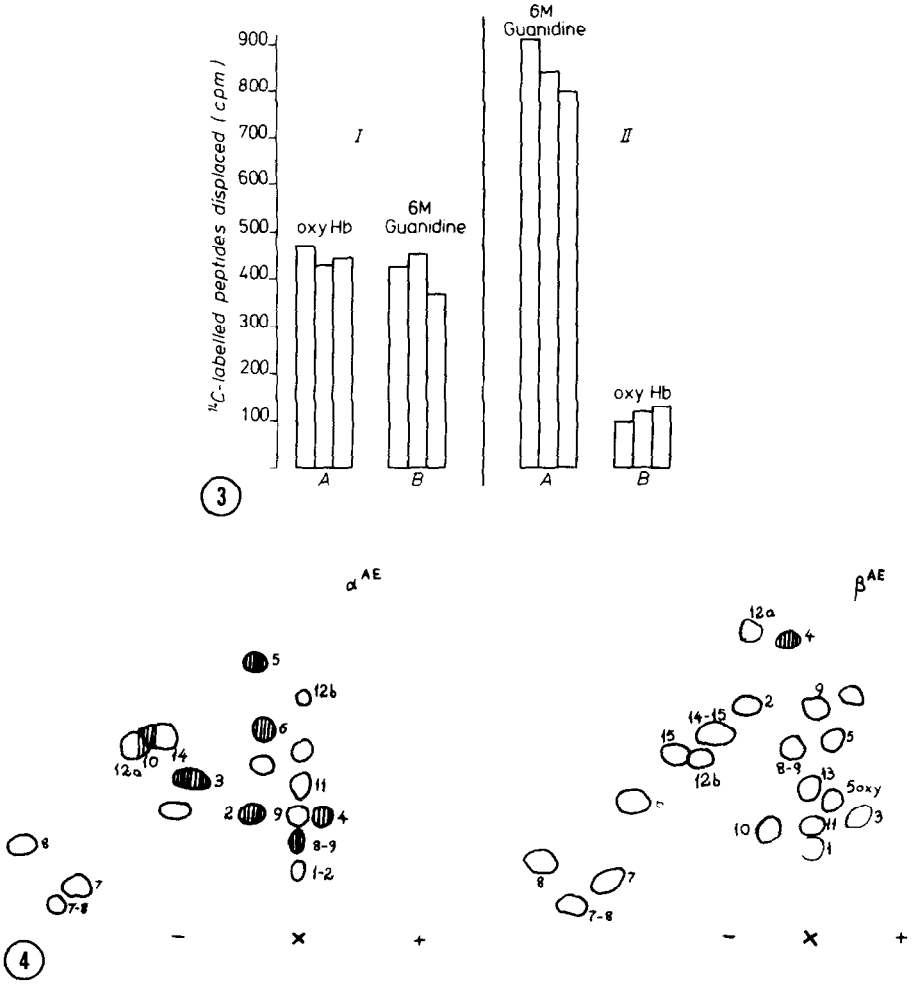

Figure 3. Aga-Hp was incubated and washed in the same conditions as described in Fig. 2. To a first sample (I) of 2ml of sedimented Aga-Hp bound to peptides we added 2 ml of oxy Hb (25 μ M) (IA) and subsequently 2 ml of 6 M Guanidine (IB). To a second sample (II) of Aga-Hp addition of 2 ml of 6 M Guanidine (IIA) was prior of the addition of oxy Hb (25 μM) (IIB). Each sample was assayed in triplicate.

Figure 4. Map of tryptic peptides from aminoethylated α and β chains. Peptides eluted from Aga-Hp by addition of oxy Hb are shaded vertically.

It must be pointed out that in a recent paper (24) it was reported that Trp-C3 (37) β becomes inaccessible in the complex formation of haptoglobin with hemoglobin. This tryptophan is a part of the β chain peptide (βT_4), which is bound specifically to Aga-Hp.

Work is in progress to characterize more precisely the contacts between Hp and Hb.

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