A doubly cross-linked human hemoglobin effect of cross-linking between different subunits.

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Abstract

Human deoxyhemoglobin cross-linked with trimesyl tris(3,5-dibromosalicylate) produces the previously reported cross-linked hemoglobin in which the epsilon amino groups of the two beta chain 82 lysyl residues are joined by a trimesyl bridge. Further specific modification of this protein directed to the alpha subunits with bis(3,5-dibromosalicyl)fumarate gives a doubly cross-linked material in which the epsilon-amino groups of the two alpha chain 99 lysyl residues are now joined by a fumaryl bridge. The singly cross-linked beta chain species binds oxygen cooperatively with a high oxygen affinity (P50 = 4.8 torr at pH 7.4). The addition of the second cross-linking reduces the oxygen affinity to 15.9 torr, which compares with 13.0 torr for the singly cross-linked alpha chain species. The doubly cross-linked hemoglobin retains significant cooperativity with a Hill coefficient of 2.3 compared with 3.0 for unmodified hemoglobin. Because some of the groups responsible for the Bohr effect are acylated, this doubly cross-linked hemoglobin exhibits 25% of the normal Bohr effect and less than 20% of the normal chloride effect. The use of two distinct cross-links within the same tetramer provides a material for physical and structural analysis as well as for further modifications for specific applications. The results indicate that the cross-link introducing the lowest oxygen affinity in the two singly cross-linked species appears to control the overall affinity in this doubly cross-linked species.