same time a significant, but less pronounced, decrease of their PP–ATP exchange activity was observed. Proteins, non-precipitable at pH 5.1, appeared during the incubation, and from the incubation mixture a nucleotide-free enzyme preparation could be obtained by the same method, which was used for the preparation of the S-protein. The transfer activity per mg protein was considerably higher in this S-protein from the incubated pH-5 fraction than in the S-protein obtained from the pH-5 supernatant[2]. At the same time, however, a higher activity of PP–ATP exchange was observed (Table I).

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A comment on the pH-dependent dissociation of haemoglobins

It is known that normal adult human haemoglobin (Hb-A) dissociates reversibly both at low pH (5–5.5)[1] and high pH (11–11.2)[2] into units having half the molecular weight of the parent molecule, and evidence has been adduced which indicates that the dissociation is asymmetric with respect to the four peptide chains ($\alpha_2\beta_2$) in the molecule[3,4].

These observations have a bearing on the question of the different resistance of Hb-F (foetal haemoglobin) and Hb-A to denaturation by alkali. Under selected conditions[5] (pH about 12.8) the reaction follows apparent first-order kinetics with $t_{1/2}$ values (*i.e.* time for 50 % conversion to alkaline haematin) at 20° of 11 sec and 1030 sec for Hb-A and Hb-F respectively. A mixture of the two species thus gives a first-order reaction plot consisting of two linear components with widely differing slopes, joined by an intermediate curved region.

Hb-F—if its behaviour is comparable with that of Hb-A—must have dissociated into half-molecules, α_2^A and γ_2^F, well below this pH, and Hb-A into α_2^A and β_2^A; the α-chains are reported to be common to both species[6,7]. Since Hb-A is wholly labile to alkali, both the α_2^A and β_2^A chains must be rapidly denatured. It seems therefore to follow that the alkali resistance of Hb-F is a property of the γ_2^F units. Furthermore, if it indeed consists of equal proportions of alkali-labile and alkali-resistant chains, there should be a marked change in slope in its denaturation-rate plot, corresponding to the presence of a resistant portion amounting to 50 % of the total Hb-F, irrespective of

Abbreviations: Hb-A, normal adult human haemoglobin; Hb-F, human foetal haemoglobin.

any further dissociation that the half-molecules may undergo. This is not in fact observed.

Two possible explanations present themselves: firstly, that Hb-F may not dissociate in the same way as Hb-A; secondly, that the dissociation equilibrium is a slow one, so that under the stated conditions denaturation has occurred before dissociation. In order to investigate the first of these possibilities, cord-blood haemoglobin (75 % Hb-F, 25 % Hb-A) was dialysed against 0.1 *M* Na_2CO_3 (pH 11.1) and sodium phosphate buffer (pH 11.6, I 0.22) and examined in the ultracentrifuge at a protein concentration of about 0.7 %. Molecular-weight determinations were made by the method of ARCHIBALD[8], according to procedures used by CHARLWOOD[9]. The partial specific volume of haemoglobin was taken as 0.749. At pH 11.1 a $S_{20,w}$ value of 2.42 with an attendant molecular weight of 53,000 was obtained, corresponding evidently to dissociation of the Hb-A portion only. On the other hand, at pH 11.6 (at which the solution fully retains the spectroscopic characteristics of haemoglobin for some days) the $S_{20,w}$ value was 2.20 and the molecular weight 32,500, indicating that complete dissociation had occurred. The dissociation equilibrium of Hb-F is thus shifted towards higher pH compared with Hb-A, but at the pH value at which the denaturation-rate measurements are made, the molecule is nevertheless dissociated.

In order to eliminate complications arising from the possibility that the dissociation process may be slow, and to ensure that the pigment subjected to denaturation was in fact dissociated, the cord-blood haemoglobin was first dialysed for 24 h at 4° against buffers of pH 11.1 to 11.6. The reaction[5] was then followed photometrically (interference filter, maximum transmission 625 mμ). No change in slope apart from the initial section corresponding to the Hb-A component was observed, and there were no significant differences from the normal-rate curve for cord blood haemolysates; extrapolation of the terminal linear part of the plot led as before to an intercept corresponding to 75 % Hb-F in the mixture.

It is difficult, therefore, to escape the conclusion either that the α-chains of Hb-A and Hb-F are not precisely identical with respect to peptide structure or haem-protein linkage, or that other factors may enter into the dissociation at high pH, in particular that it might be symmetrical rather than asymmetrical. If such is in fact the case, the results of a number of hybridisation experiments on human haemoglobins carried out at high pH would be open to re-interpretation[4,10].

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