State of methaemoglobin in normal adults and in patients with hereditary methaemoglobinaemia

Max F. PERUTZ

M.R.C. Laboratory of Molecular Biology, Hills Road, Cambridge CB2 2QH, U.K.

(Received 19 January 1981/Accepted 5 February 1981)

The predominance of valency hybrids over fully oxidized methaemoglobin in the haemolysate from a methaemoglobinaemia patient can be explained by random oxidation of the haems, followed by re-assortment and co-migration of equally charged $\alpha\beta$ -dimers in the electric field of the isoelectric-focusing gel.

Tomoda *et al.* (1979) analysed methaemoglobin in a normal haemolysate, in the haemolysate of a patient with methaemoglobinaemia and in a ferricyanide-treated normal haemolysate by isoelectric focusing on Ampholine/polyacrylamide gels. They found that the dominant portion of the oxidized haemoglobin was present as valency hybrids rather than fully oxidized methaemoglobin. They concluded that either the rate constants of oxidation of half-oxidized haemoglobin to methaemoglobin are small compared with the rate constants of oxyhaemoglobin to half-oxidized haemoglobin, or the quantity of methaemoglobin formed from half-oxidized haemoglobin is small because the half-oxidized haemoglobins as substrates do not accumulate so much. I am not sure what is meant by the second alternative.

I suspected that the distribution of ferric haems observed by Tomoda *et al.* (1979) might be due to an artifact first explained by Guidotti *et al.* (1963). Until then investigators had been puzzled why the blood of heterozygotes for HbS contained only pure HbS and HbA, but no hybrids $\alpha_2^S\beta^A\beta^S$. Guidotti *et al.* (1963) showed that, in a mixture of HbA and

HbS, tetramers and dimers are in rapid equilibrium; in an electric field dimers of equal charge co-migrate and re-associate into tetramers:

The haemolysate of the methaemoglobinaemia patient studied by Tomoda *et al.* (1979) contained 20% of oxidized HbA. Haemoglobin molecules consist of symmetrically placed $\alpha\beta$ -dimers that are equivalent. Suppose oxidation occurred randomly among the haems in the $\alpha\beta$ -dimers. If one-fifth of the haem groups are oxidized, their distribution among the dimers will be as shown in Table 1.

The agreement between observed and calculated fractions suggests that the results that Tomoda *et al.* (1979) obtained from their methaemoglobinaemia patients can be accounted for by random oxidation followed by re-assortment of equally charged dimers in the electric field. In a normal haemolysate Tomoda *et al.* (1979) found traces of half-oxidized hybrids but no fully oxidized methaemoglobin. Their scan shows that a peak of one-eighth the area of the hybrid peak would have escaped detection. They also show a photograph of the gel of their ferricyanide-oxidized haemolysate, but no scan; the

Abbreviation used: Hb, haemoglobin.

Table 1. Predicted and observed distribution of valency hybrids in the haemoglobin of a methaemoglobinaemia patient

Dimers	Probability	Percentage distribution	
		Predicted	Observed by Tomoda <i>et al.</i> (1979)*
$\alpha^2\beta^2$	$4/5 \times 4/5 = 16/25$	64	63.0
$\alpha^3\beta^2 + \alpha^2\beta^3$	$2(1/5 \times 4/5) = 8/25$	32	32.5
$\alpha^3\beta^3$	$1/5 \times 1/5 = 1/25$	4	4.6

* The fractions given here are of HbA, ignoring HbA₂.

photograph suggests that the distribution of hybrids and fully oxidized HbA molecules is similar to that of the methaemoglobinaemia patient. In each of the gels hybrids $\alpha_2^3+\beta_2^{2+}$ are about 50% more abundant than $\alpha_2^2+\beta_2^{3+}$. This is to be expected, because the haems of the α -subunit are more readily oxidized than are the haems of the β -subunit (Mansouri & Winterhalter, 1973).

References

- Guidotti, G., Konigsberg, W. & Craig, L. C. (1963) *Proc. Natl. Acad. Sci. U.S.A.* **50**, 774–783
- Mansouri, A. & Winterhalter, K. H. (1973) *Biochemistry* **12**, 4946–4949
- Tomoda, A., Imoto, M., Hirano, M. & Yoneyama, Y. (1979) *Biochem. J.* **181**, 505–507