complex like the genes of major histocompatibility complex also plays a role in conferring resistance against TB.

1. Skamene, E., *Curr. Top. Microbiol. Immunol.*, 1996, **124**, 49–63.
2. Kaledin, V., Kurunov, Y. N. and Serova, I. A., *J.N.C.I.*, 1997, **58**, 423–431.
3. Stead, W. W., Lofgren, J. P., Sener, J. W. and Reddick, W. T., *N. Engl. Med.*, 1990, **42**, 422–427.
4. Vogel, F. and Motulsky, A. G., *Human Genetics: Problems and Approaches*, Springer, Berlin, 1986, p. 88.
5. Donovick, R., Mckee, C. M., Jambur, W. P. and Rake, G., *Am. Rev. Tuberc.*, 1949, **60**, 109–120.
6. Blackwell, J. M., *Res. Immunol.*, 1989, **140**, 767–828.
7. Schurr, E., Skamene, E., Morgan, K., Chu, M. L. and Gros, P., *Genomics*, 1990, **8**, 477–486.
8. Vidal, S., Malo, D., Vogan, K., Skamere, K. and Gros, P., *Cell*, 1993, **73**, 469–485.
9. Vidal, S., Tremblay, M. L., Govoni, G., Gauthier, S., Sebastiano, G., Malo, D., Skamene, E., Oliver, S. Jothy and Gros, P., *J. Exp. Med.*, 1995, **182**, 655–666.
10. Malo, D., Hu, J., Skamene, E. and Schurr, E., *Anim. Biotechnol.*, 1994, 173–182.
11. Miller, S. A., Dykes, D. D. and Polesky, H. F., *Nucleic Acids Res.*, 1998, **16**, 1215.
12. Liu, J., Fujiwara, T. M., Buu, N. T., Sanchez, F. O., Cellier, M., Paradis, A. J., Frappier, B., Skamene, E., Gros, P. and Moryan, K., *Am. J. Hum. Genet.*, 1995, **56**, 845–853.
13. Cellier, M., Govoni, G. , Vidal, S., Kwan, T., Groulx, N., Liu, J. and Sanchez, F., *J. Exp. Med.*, 1994, **180**, 1741–1752.
14. Levin, H. A., *J. Dairy Sci.*, 1989, **72**, 1334–1342.
15. Govonic, G., Vidal, S., Gautheier, S., Skamene, E., Malo, D. and Gros, P., *Infect. Immunol.*, 1996, **64**, 2923–2929.
16. Lang, T., Prina, E., Sibthorpe, D. and Blackwell, J. M., *Infect. Immunol.*, 1997, **65**, 380–386.

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Spectrin exhibits chaperone-like activity

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Erythroid spectrin, a cytoskeletal protein, inhibits refolding of denatured heme enzyme, horseradish peroxidase (HRP). The denatured enzyme after 30 min refolding in the absence and presence of spectrin showed significant differences in the enzyme activity. The enzymic activity of HRP decreased in the presence of spectrin when compared with the activity in absence of spectrin. This inhibitory effect of spectrin is abolished when it was preincubated with magnesium-ATP indicating that spectrin exhibits chaperone-like activity.

SPECTRIN is the major structural protein of the erythrocyte membrane skeleton. It is an elongated $\alpha\beta$-heterodimer with large molecular weight of approximately 460,000 Dalton[1,2]. Spectrin isoforms have been identified in a wide variety of nonerythroid cells where they are also localized predominantly along cellular plasma membranes[2]. Spectrin binds to a wide range of ligands starting from metal ions like calcium and molybdenum, fatty acids and phospholipids to proteins like actin, ankyrin and calmodulin[1,2]. The major function of spectrin is presumed to establish the cytoskeletal network that provides mechanical strength to cells. However, its capability to interact with a wide range of different ligands and its abundance also in nonerythroid tissues suggests that spectrin might have a role to play in other functions as well. Like some of the heat-shock proteins[3,4] and the eye lens structural protein α-crystallin[5], spectrin interacts with phospholipid membranes[6,7]. Spectrin binds to fatty acids and many other hydrophobic ligands[8–10] which is also seen in chaperone proteins that bind hydrophobic fluorescent probes like ANS and bis-ANS (ref. 11). The heat-shock proteins and a few structural proteins, e.g. eye lens α-crystallin have been shown to exhibit chaperone-like activity by interacting with the denatured proteins[12–14]. Also, recently the cytoskeletal protein, tubulin, has been shown to exhibit chaperone-like activity in preventing the aggregation of other proteins[15]. In view of these properties of the erythroid spectrin, we tried to investigate if spectrin can act as molecular chaperone by interacting with the unfolded proteins. We chose a heme enzyme, horseradish peroxidase (HRP) for such a study because of (i) the abundance of haemoglobin in erythrocytes that was earlier known to interact with spectrin[16] and (ii) a monomeric protein whose folding pathway has been worked out[17,18] and its activity can be readily determined in a continuous assay.

The enzyme activity of HRP (Type VI, Sigma) was measured according to the ABTS assay method[18] by following its change in absorbance at 405 nm in 50 mM phosphate-citrate buffer, pH 5.0. Spectrin dimers were purified from ovine erythrocyte ghost membranes following published protocols[19,20].

HRP (100 µg/ml) was incubated with 6 M guanidinium chloride (GdmCl, Sigma) for 2.5 h at 25°C for denaturation. Refolding was initiated by 40-fold dilution of the denatured HRP in 50 mM Tris-HCl, pH 7.8 with or without dimeric spectrin. After 30 min of refolding at 25°C, the enzyme activity was measured by ABTS assay method. The extent of reactivation of the denatured HRP

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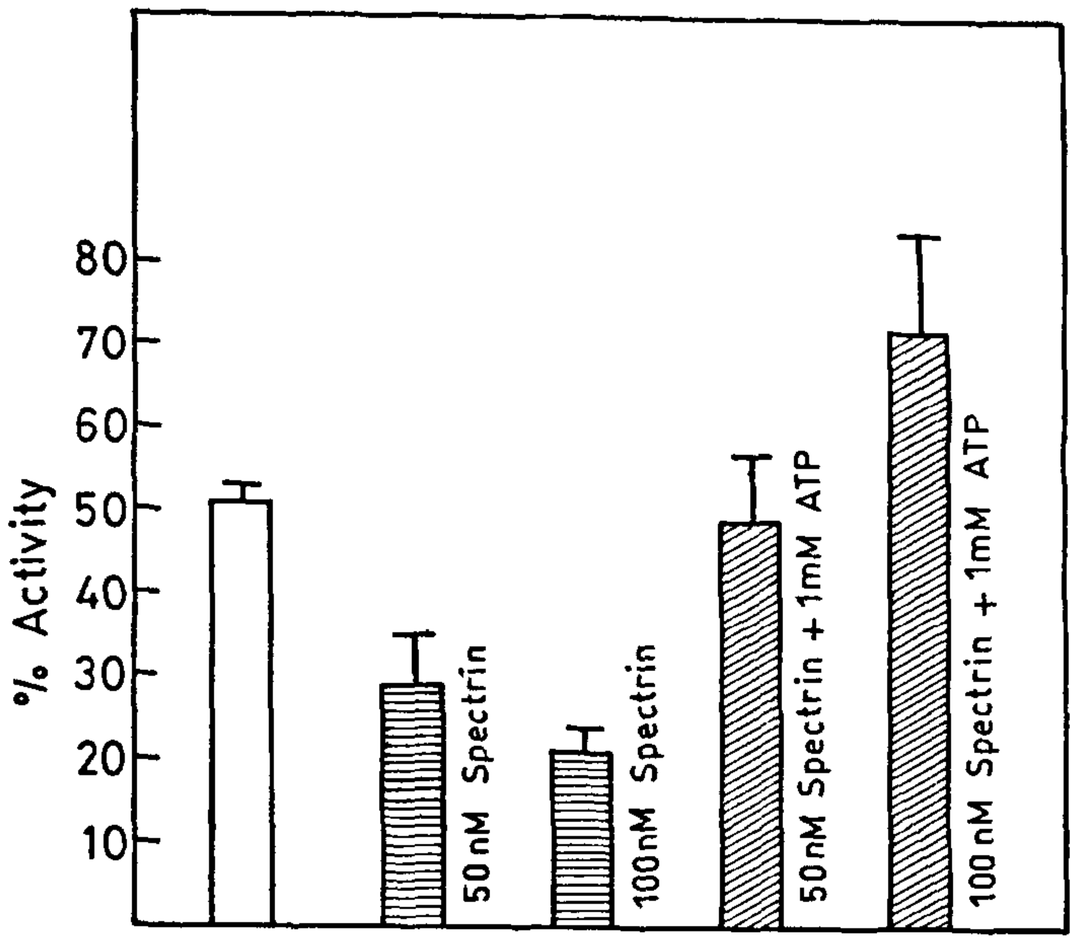

Figure 1. Histogram representation of the extent of reactivation in percentage keeping the activity of the native enzyme as 100%. The empty bar represents the activity of denatured enzyme after 30 min refolding in absence of spectrin. Bars shaded with horizontal lines represent the enzyme refolded in presence of spectrin. Bars shaded with slanted lines represent the enzyme refolded in presence of spectrin preincubated with 1 mM ATP, 10 mM $MgCl_2$. The error bars represent Standard Error of Mean for 10 independent experiments.

after refolding was determined assuming the activity of the native enzyme to be 100%.

Figure 1 shows a histogram representation of the data on the reactivation of the denatured HRP after 30 min refolding in the absence and presence of two different concentrations, 50 nM and 100 nM, of dimeric spectrin in the reconstitution buffer. Reactivation of the enzyme was found to be inhibited in the presence of spectrin. When refolding of denatured HRP was carried out in the presence of 50 nM spectrin preincubated with 1 mM ATP in 10 mM $MgCl_2$, there was no inhibition in the reactivation of the denatured enzyme. At a still higher spectrin concentration of 100 nM, there was enhancement in the extent of reactivation of the enzyme in the presence of 1 mM ATP in 10 mM $MgCl_2$ when compared with that in the absence of spectrin (Figure 1). The native enzyme does not show any change in the enzyme activity in presence of spectrin, indicating that spectrin does not take part in the enzyme assay and the effect observed is only due to the inhibition in refolding of denatured HRP. Similarly, presence of 10 mM $MgCl_2$ in the reconstitution buffer was also found to have no effect on the extent of refolding of the denatured HRP (data not shown).

Results clearly show that spectrin inhibits refolding of denatured horseradish peroxidase in the absence of magnesium-ATP. We have also shown that spectrin preincubated with magnesium-ATP does not show such inhibition. Our results show striking similarity with the *Escherichia coli* chaperonin, GroEL, which binds with high affinity to the completely denatured form of lactate dehydrogenase while such a complex between the GroEL and the denatured protein is destabilized by the binding of magnesium-ATP (ref. 21). Spectrin being the major component of the erythrocyte cytoskeleton, the chaperone-like activity might be an important function of spectrin in interacting with the non-native forms of hemoproteins in particular, e.g. hemoglobin, if not all proteins.

1. Bennett, V., *Curr. Opin. Cell Biol.*, 1990, **2**, 51–56.
2. Hartwig, J., *Protein Profile*, 1994, **1**, 715–778.
3. Adamska, I. and Kloppstech, K., *Eur. J. Biochem.*, 1991, **198**, 375–381.
4. Lavoie, J. N., Hickey, E., Weber, L. A. and Landry, J., *J. Biol. Chem.*, 1993, **268**, 24210–24214.
5. Mulders, J. W. M., Stockkermans, J., Leunissen, J. A. M., Benedetti, E. L., Bloemendal, H. and de Jong, W. W., *Eur. J. Biochem.*, 1985, **152**, 721–728.
6. Cohen, A., Liu, S., Derick, L. and Palek, J., *Blood*, 1985, **68**, 920–926.
7. Diakowski, W. and Sikorski, A. F., *Biochemistry*, 1995, **34**, 13252–13258.
8. Kahana, E., Pinder, J. C., Smith, K. S., and Gratzer, W. B., *Biochem. J.*, 1992, **282**, 75–80.
9. Chakrabarti, A., *Biochem. Biophys. Res. Commun.*, 1996, **226**, 495–497.
10. Majee, S. and Chakrabarti, A., *Biochem. Biophys. Res. Commun.*, 1995, **212**, 428–432.
11. Das, K. P., Petrash, J. M. and Surewicz, W. K., *J. Biol. Chem.*, 1996, **271**, 10449–10452.
12. Hendrick, J. P. and Hartl, F-U., *Annu. Rev. Biochem.*, 1993, **62**, 349–384.
13. Buckle, A M., Zahn, R. and Fersht, A. R., *Proc. Natl. Acad. Sci. USA*, 1997, **94**, 3571–3575.
14. Horwitz, J., *Proc. Natl. Acad. Sci. USA*, 1992, **89**, 10449–10453.
15. Guha, S., Manna, T. K., Das, K. P. and Bhattacharyya, B., *J. Biol. Chem.*, 1998, **273**, 30077–30080.
16. Cassoly, R., *FEBS Lett.*, 1978, **85**, 357–360.
17. Pappa, H. S. and Cass, A. E. G., *Eur. J. Biochem.*, 1993, **212**, 227–235.
18. Haque, M. E., Debnath D., Basak, S. and Chakrabarti, A., *Eur. J. Biochem.*, 1999, **259**, 269–274.
19. Gratzer, W. B., *Methods Enzymol.*, 1985, **85**, 475–480.
20. Majee, S., Dasgupta, D. and Chakrabarti, A., *Eur. J. Biochem.*, 1999, **260**, 619–626.
21. Badcoe, I. G., Smith, C. J., Wood, S., Halsall, D. J., Holbrook, J. J., Lund, P. and Clarke, A. R., *Biochemistry*, 1991, **30**, 9195–9200.

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