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Studies on Yeast Invertase

III. Inhibition by Ag^+. Model Experiments with Egg Albumin *

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Yeast invertase is strongly inactivated by Ag+ and certain other metal ions. Several reasons, *i. a.* the fact that iodine-treated invertase is rather insensitive to Ag+, support the assumption that Ag+ is bound to HS-groups of the protein. Since the Ag-inhibition of the enzyme is strongly dependent on pH in the range 4—7 it is concluded that certain groups, probably imidazole groups, of the enzyme interfere with the HS-groups, rendering them unavailable to Ag+ in acid solution where the imidazole groups are protonated. For a comparison the Ag-binding capacity of egg albumin has been determined, directly as well as indirectly, using invertase for the Ag determination. The experiments support the view that Ag+ is bound to egg albumin principally if not exclusively to HS-groups. The binding of Ag+ by egg albumin is practically independent of pH in the pH-range mentioned, *i. e.* the HS-groups of egg albumin are, in contrast to those of invertase, freely available to Ag+ in this range. In agreement with earlier investigations the number of HS-groups in an egg albumin molecule (M = 44 000) is found to be 5.

In earlier investigations from this laboratory it was found [1,2] that, to explain the inhibition of yeast invertase by various mercury compounds, the assumption of a reaction of Hg with sulfhydryl groups of the enzyme appears to be consistent with all known facts. The enzyme is also strongly inhibited by Ag^+ (and several other heavy metal ions) and *a priori* one would be inclined to regard HS-groups as responsible also for these inhibitions. However, the inhibition by Ag^+ and certain other metal ions show a dependence on the acidity of the medium which seems to indicate a reaction of the metal ions with groups of the enzyme [3–9] having pK-values around 7. Several reasons, among others the fact that the complexing of Ag^+ by histidine [8] shows the same dependence on pH as the inhibition of the enzyme, suggest a participation of imidazole groups in the reaction of the enzyme with Ag^+. However, the affinity of histidine, not to speak of histamine [2] or imidazole [6], for Ag^+

* Part II, *cf.* Ref.[2]

is very weak compared to that of the enzyme, and if the assumption is correct that the Hg-inhibition of the enzyme is mediated by HS-groups it seems impossible to believe that Ag^+ with its very high affinity for sulfhydryl groups [6] should react preferentially with imidazole groups of invertase.

Investigations on iodine-treated invertase [2] appear to furnish an explanation of the somewhat conflicting experimental evidence. Treatment of yeast invertase with moderate amounts of iodine for a short time results in the formation of a modified enzyme "I-invertase", having an activity about 55 % of that of the native enzyme. Although iodine may conceivably act in several different ways on a protein, the simplest explanation is an oxidation of HS-groups. In the case of yeast invertase this assumption is strongly supported by the fact that I-invertase is much less sensitive to Ag-, Hg- and other inhibiting metal ions than the native enzyme [2]. In fact, the affinity of I-invertase for Ag^+ is so low that the Ag-inhibition of this modified enzyme may well be explained as a reaction of the metal ion with imidazole groups of the protein.

The experiments on the metal ion inhibition of invertase and I-invertase appear to show conclusively that HS-groups are in the first place responsible for the high affinity of the native enzyme for Hg, Ag and certain other metal ions. However, it remains to explain the pH-dependence of the Ag-inhibition of the enzyme. A tentative explanation has been given [2], involving the assumption of some sort of interaction, in the native enzyme, of the HS-groups with adjacent imidazole groups, possibly hydrogen bonding. Under all circumstances we have to conclude that in acid solution (pH $<$ *ca.* 5) the HS-groups of native invertase are unavailable ("masked") to the Ag ion. However, with pK $\sim$ 6.7 the native enzyme is converted reversibly to a structurally different form in which the HS-groups are available to Ag^+. If the groups with pK $\sim$ 6.7 are imidazole groups, which seems probable, it must be concluded that in the active enzyme these groups are protonated, but that loss of the proton is followed by a more or less radical change of protein structure, accompanied by reversible inactivation of the enzyme and by unmasking of the HS-groups. The reactivity of the HS-groups toward Ag and certain other metal ions will be determined by the presence or absence of the proton on the imidazole groups. Thus, in the interaction with Ag^+ (Cu^{++} etc.) the HS-groups of native invertase will have an apparent pK value of ca. 6.7. This is in accord with the assumption [3] that the Ag-binding groups of invertase are identical with the "acidic" group with pK $\sim$ 6.7, assumed by Michaelis [10] to explain the alkaline branch of the activity-pH-curve of invertase.

In order to compare the binding of Ag^+ by invertase and by a well-known, "ordinary" protein, experiments have been carried out with egg albumin which is known to contain more or less reactive HS-groups. Since this protein is easily available and can be obtained in a high degree of purity, it has been the object of a large number of investigations. The molecular weight has been determined by several authors; the values differ somewhat but a value of M = 44 000 should not be far from correct [11,12]. This value has been used in the following.

The number of HS-groups in an egg albumin molecule (or its cysteine content, respectively) has been determined by means of many different methods. Mirsky and Anson [13] and Anson [14] compared the action of several HS-

reagents; oxidation with ferricyanide under specified condition was considered to give reliable values. Native egg albumin was found to be inert to this reagent, but the denatured protein reduced ferricyanide: 10 mg protein corresponded to 0.001 mmole of the oxidant, *i.e.* one molecule of egg albumin was found to contain ca. 4.4 HS-groups. Iodine and iodoacetamide were found to react with the native protein, other HS-reagents generally only after denaturation. Egg albumin, treated with iodine, iodoacetamide or formaldehyde did not reduce ferricyanide. The authors conclude that native egg albumin has "free and accessible but relatively unreactive HS-groups which can react with iodine and acetamide despite the fact that they do not react with ferricyanide, porphyrindin or nitroprusside". Greenstein [15] and Brand and Kassell [16] likewise state that native egg albumin does not react with porphyrindin; in the denatured protein the HS-groups are oxidized. Values quoted by Greenstein and Jenrette [17] tend to show that at least 4, probably 5 HS-groups are present in an egg albumin molecule. Hellerman *et al.*[18], using iodosobenzoate, also give the number of HS-groups in egg albumin as 5. MacDonnell, Silva and Feeney [19] applied *p*-chloromercuribenzoate to egg albumin. They found, contrary to Anson's results, that 3 HS groups are reactive already in the *native* protein and 4 after denaturation. With a spectrometric method Boyer [20] found that 4.0 moles *p*-chloromercuribenzoate are bound at pH 4.6 by one egg albumin molecule, whereas Benesh, Lardy and Benesh [21] using an amperometric titration method with silver found 5 HS-groups per protein molecule.

Anson [14] found that iodine destroys the HS-groups of egg albumin. It seems to be generally assumed that the oxidation of protein HS-groups results in the formation of disulphide linkages. It should be noted, however, that according to Fraenkel-Conrat [22] the HS-groups of tobacco mosaic virus protein on treatment with iodine give stable sulphenyl iodide groups. "Overoxidation" of thiol groups to levels higher than the disulphide must also be considered. It should be emphasized that formation of disulphide linkages on treatment of proteins with iodine requires a sterically favourable position of the thiol groups, especially if intramolecular disulphide linkages are formed (*cf.* Ingram [23]).

EXPERIMENTAL

Materials

Invertase. The enzyme preparations used in the experiments were partly purified from autolysates of baker's yeast. The solutions were thoroughly dialyzed before use.

I-Invertase was prepared as described earlier [2]. The solutions were dialyzed with particular care to remove all iodide ions.

Buffer. Acetate-acetic acid buffers were used in all experiments. The buffer concentration was kept so low that complexing av Ag^+ by the acetate ion did not cause any serious decrease of the calculated Ag-ion concentration [5]. In the assay mixtures used for determination of enzymatic activity the concentration of the acetate ion was 0.01 N and in the solutions used for electrometric Ag-determination 0.05 N.

pH was determined by means of the glass electrode in all assay mixtures.

Egg albumin. Crystallized egg albumin was prepared according to the method of Sørensen and Høyrup [24] with minor modifications: *Albumin I* was recrystallized once, *Albumin II* three times. All albumin solutions were dialyzed thoroughly before use. The two albumin preparations behaved identically in all experiments; consequently the observed effects can be ascribed to egg albumin itself. *Albumin III* was a commercial pre-

paration; dialyzed solutions were used for a comparison in some experiments without further purification. Qualitatively it behaved as the crystalline preparations but its Ag-binding capacity was somewhat lower.

Methods

Invertase activity was determined polarimetrically as described earlier [4]. The relative activity (RA) refers to the activity of the same amount of enzyme without inhibitor at pH 4.5. The RA-values are means of 4—5 polarimetric determinations at different reaction times. All enzymatic determinations were carried out in 100 ml volume, containing 4.75 g saccharose. Temperature 30°C.

Electrometric determination of the Ag+ ion was carried out according to the simple method originally described by v. Euler and Svanberg [25]. These authors determined the Ag-binding capacity of various substances in order to find out the chemical nature of the Ag-binding groups of enzymes. They found, among other things, that egg albumin binds Ag+ very strongly. In the present investigation the electrometric determinations were carried out at several different pH-values; this is necessary since the Ag-binding capacity of invertase varies strongly with pH.

RESULTS

Binding of Ag^+ by egg albumin, determined electrometrically

The electrode vessels contained 5×10^{-5} M silver nitrate, acetate buffer (0.05 N Ac^-); to one of the vessels egg albumin solution was added. Total volume 20 ml, temperature 20°C. The constancy of the potential differences between the silver electrodes was checked by repeated measurements during at least 30 min. The Ag^+ concentrations were calculated in the conventional way (Table 1).

Table 1. Binding of Ag+ by crystalline egg albumin. $[Ag_{tot}] = 5 \times 10^{-5}$.

mg Albumin II in 20 ml	$[Ag^+] \times 10^5$		
	pH 4.5	pH 5.3	pH 5.95
0	5.00	5.00	5.00
2.82	3.55	3.25	2.74
5.64	1.94	1.33	1.12
8.46	0.44		

Fig. 1 shows the number of Ag^+ ions bound per molecule of egg albumin at different pH-values. For a comparison Fig. 2 shows corresponding results [8] for histidine. The Ag-binding capacity of histidine varies very strongly with pH in the range 4.5—6; the explanation is that H^+ competes with Ag^+ for the imidazole ($pK \sim 6.1$). In contrast, the Ag-binding of egg albumin is nearly independent of pH in the same range, showing that the groups essentially responsible for the binding of Ag^+ to the protein do not change their charge appreciably in the pH-range mentioned. This is obviously in accord with the assumption, that the binding of silver to egg albumin is mediated principally by HS-groups of the protein. The weak dependence of the silver-

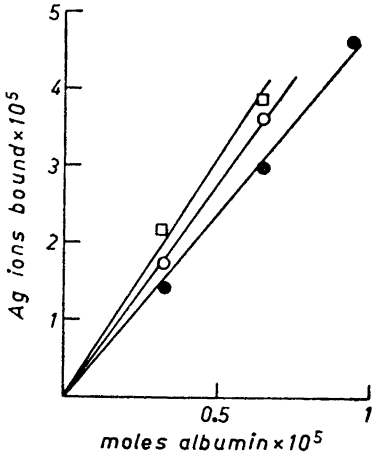

Fig. 1. Number of Ag ions (volume 1 000 ml) bound by egg albumin at pH 4.5 (●) pH 5.3 (○), and pH 5.95 (□).

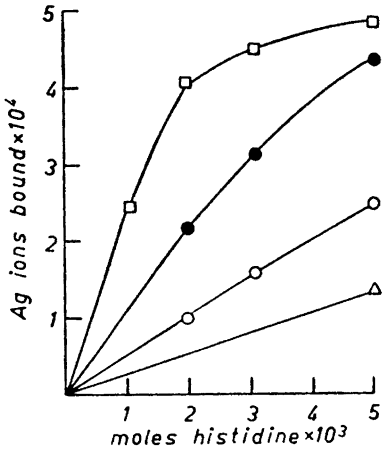

Fig. 2. Number of Ag ions (volume 1 000 ml) bound by histidine at pH 4.5 (△), pH 4.8 (○), pH 5.3 (●), and pH 5.95 (□).

binding of egg albumin upon pH may intimate that other groups than HS-groups are, to some small degree, responsible for the binding of silver ions, and that these groups have pK-values in the range 4—8.

In Fig. 1 the molar concentration of albumin has been calculated, assuming the molecular weight to be 44 000. The practically straight lines of the figure show that the affinity of egg albumin for Ag^+ is very high and the number of Ag^+ ions bound by one molecule of egg albumin can be calculated fairly accurately. One albumin molecule binds:

at pH 4.5	4.8 Ag^+ ions
at pH 5.3	5.5 » »
at pH 5.95	6.2 » »

Similar experiments were also carried out at the 10-fold Ag^+ concentration, 5×10^{-4} M. These experiments were less well reproducible; sometimes a precipitate was formed when protein and silver solutions were mixed. Nevertheless, as seen from Table 2, the results are in good agreement with those

Table 2. Binding of Ag+ by crystalline egg albumin. $[Ag_{tot}] = 5 \times 10^{-4}$.

mg Albumin II in 20 ml	$[Ag^+] \times 10^4$	
	pH 4.5	pH 5.3
0	5.00	5.00
14.1	—	3.68
28.2	2.89	2.58
42.3	1.72	1.64
56.4	0.84	0.65

Table 3. Binding of Ag+ by commercial egg albumin. $[Ag_{tot}] = 5 \times 10^{-5}$.

mg Albumin III in 20 ml	[Ag+] × 10^5			
	pH 4.5	pH 5.3	pH 5.7	pH 5.95
0	5.00	5.00	5.00	5.00
2.35	3.57	3.72	3.31	3.57
4.70	—	2.87	2.47	2.27
7.05	1.94	1.77	1.40	1.20
11.75	0.72	—	—	—
23.50	—	0.24	—	—

obtained in more diluted solutions: The metal ion is bound very strongly and the amounts bound at pH 5.3 are only slightly larger than those bound at pH 4.5. About 6 Ag ions are bound per molecule of albumin. This value is somewhat higher than that found in the experiments in more diluted solution. The explanation may be that at higher concentrations more Ag^+ ion is bound by non-HS-groups of the albumin.

Corresponding determinations were also carried out with the albumin preparation III. The lower Ag^+ concentration was used. Table 3 shows that the Ag-binding capacity of albumin III is slightly lower than that of albumins I and II; otherwise the results with all three preparations are very similar.

Binding of Ag^+ to egg albumin, determined by inhibition experiments with invertase

The Ag-binding capacity of egg albumin (or any other Ag-binding substance, not interfering with invertase) may be determined by studying the inhibition of invertase by Ag^+ in the presence of the substance in question. These deter-

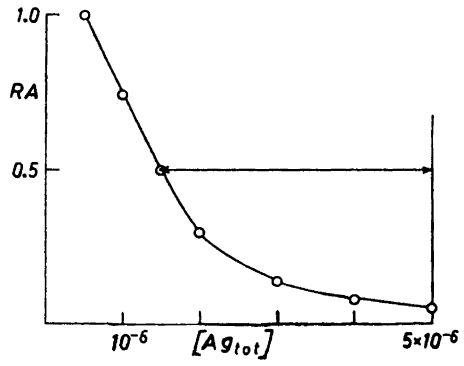

Fig. 3. Relative activity (RA) of invertase at various concentrations, $[Ag_{tot}]$, of silver ions; pH 5.3.

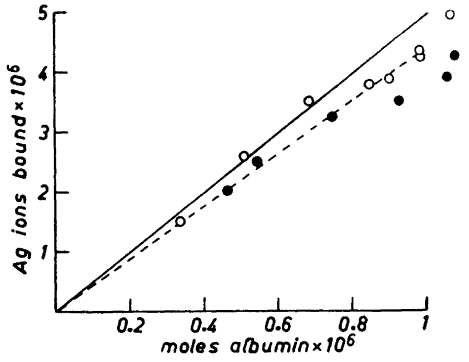

Fig. 4. Number of Ag ions in 1 000 ml bound by egg albumin at pH 5.3 as calculated from inhibition of invertase. Crystalline egg albumin (○), commercial preparation of albumin (●).

minations may be carried out at considerably lower Ag^+ concentrations than the electrometrical determination described above.

The invertase used for experiments of this kind need not be of any extremely high purity. The relative activity of a certain amount of enzyme is determined at constant pH and various Ag^+ concentrations. In one series of experiments the curve shown in Fig. 3 was obtained. A certain RA-value corresponds to a certain concentration of free Ag^+ ions, $[Ag_{free}]$, in equilibrium with $[Ag_{bound}]$, *i. e.* the concentration of Ag bound to invertase and, usually, to impurities in the invertase solution. For instance, RA = 0.06 corresponds to $[Ag_{tot}] = [Ag_{free}] + [Ag_{bound}] = 5 \times 10^{-6}$. If an experiment is carried out at this total Ag-concentration in the presence of, *e.g.* 3 mg of egg albumin, part of the silver will be bound to the albumin and a higher RA-value will be obtained, *e.g.* RA = 0.5. It is seen from Fig. 3 that this RA-value corresponds to a sum of free Ag-ion and Ag bound to substances in the enzyme solution $= 1.5 \times 10^{-6}$. Since the volume of the solution is 100 ml, we find that 3.5×10^{-7} g-ions Ag^+ have been bound by 3 mg egg albumine or 0.68×10^{-7} albumin molecules, *i.e.* one albumin molecule has bound 5 silver ions.

It should be added that experiments of this kind can give clearcut results only if *a*) the inhibition of the enzyme by Ag+ is independent or practically independent of the time of incubation of enzyme and inhibitor, and *b*) the binding of Ag^+ by the tested substance (egg albumin) is (practically) independent of any incubation time. If these requirements are not fulfilled the determination of the RA-values will be impossible or precarious. In the case of yeast invertase and Ag+ and of egg albumin and Ag+ the binding of the metal ion occurs with unmeasurably high velocity, and incubation of the proteins with Ag+ for reasonable times does not increase materially the uptake of the metal ion.

Using the method outlined above a series of experiments was carried out at pH 5.3 and $[Ag_{tot}] = 5 \times 10^{-6}$ in the presence of various amounts of recrystallized egg albumin (Table 4). The concentration of Ag bound by the albumin was estimated by means of the curve shown in Fig. 3. Fig. 4 shows the number of bound Ag-ions *vs* the number of albumin molecules. The curve is a practi-

Table 4. Relative activity (RA) of invertase at pH 5.3 and $[Ag_{tot}] = 5 \times 10^{-6}$ in the presence of crystalline egg albumin.

Albumin I mg/100 ml	RA	$[Ag_{tot}] \times 10^6$ corresponding to RA	$[Ag] \times 10^6$ bound by albumin
0	0.064	5.0	0
1.48	0.114	3.5	1.5
2.22	0.214	2.4	2.6
2.96	0.479	1.5	3.5
2.96 *	0.479	1.5	3.5
3.70	0.647	1.2	3.8
4.00	0.721	1.1	3.9
4.28	0.875	0.75	4.25
4.28 *	0.923	0.65	4.35
4.73	1.00	0	5.00

* Albumin incubated with Ag+ during 30 min before determination of RA.

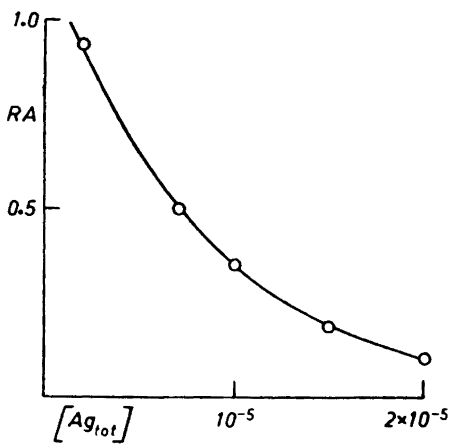

Fig. 5. Relative activity (RA) of invertase at various concentrations, [Ag_{tot}], of silver ions; pH 4.5.

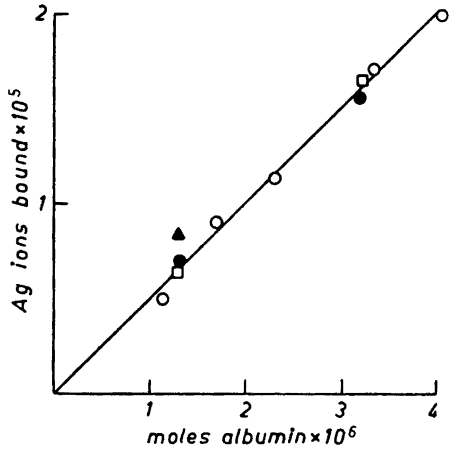

Fig. 6. Number of Ag ions in 1 000 ml bound by egg albumin at pH 4.5 as calculated from inhibition of invertase. Crystalline egg albumin I (○), crystalline egg albumin II (□), crystalline egg albumin II, treated with N NaOH (▲), crystalline egg albumin, treated with pepsin (●).

cally straight line, showing that Ag is bound very strongly by the albumin. One molecule of egg albumin binds 5.0 Ag ions; this number is in reasonably good agreement with that obtained (5.5) by electrometrical Ag-determination at the same pH.

In Fig. 4 have also been plotted corresponding values obtained with the commercial egg albumin preparation III. The results are qualitatively the same as with the crystalline albumin preparation but, as in the electrometric determinations, the values are somewhat lower with the commercial preparation.

Similar experiments with crystalline egg albumin were carried out at pH 4.5. The same invertase preparation was used and the RA-values in absence of egg albumin are plotted in Fig. 5 *vs.* [Ag_{tot}]; a comparison with the corresponding curve for pH 5.3 (Fig. 3) shows the much weaker inhibition by Ag^+ in the more acid solution: 50 % inhibition at pH 5.3 is caused by [Ag_{tot}] = 1.5×10^{-6} N Ag^+, at pH 4.5 by [Ag_{tot}] = 7×10^{-6} N.

The experiments in presence of egg albumin were carried out at [Ag_{tot}] = 2×10^{-5} and the amounts of Ag bound were calculated as above (Table 5). The native albumin preparations I and II were used as well as albumin II treated with alkali (N sodium hydroxide, 120 h, 20°). A peptic hydrolysate of egg albumin was also tested: Heat-coagulated albumin was treated with a small amount of pepsin at pH 1.6 (sulphuric acid). The coagulum was dissolved after *ca.* 5 h. The Ag-binding capacity of the hydrolysate was determined after 24 and 72 h, respectively. In Fig. 6 the number of bound Ag-ions have been plotted *vs.* the number of albumin molecules. The figure shows that all albumin preparations have the same Ag-binding capacity.

Table 5. Relative activity (RA) of invertase at pH 4.5 and $[Ag_{tot}]$ 2×10^{-5} in the presence of various albumin preparations.

Albumin preparation	mg Albumin in 100 ml	RA	$[Ag_{tot}] \times 10^5$ corresponding to RA	$[Ag] \times 10^5$ bound by albumin
—	0	0.100	2.00	0
Albumin I	4.44	0.168	1.50	0.50
	7.40	0.294	1.10	0.90
	10.36	0.420	0.86	1.14
	14.80	0.846	0.30	1.70
Albumin II	5.64	0.206	1.35	0.65
	14.10	0.804	0.33	1.67
Albumin II stored in N NaOH	5.65	0.287	1.15	0.85
	28.2	1.00	0	2.00
Albumin treated with papain	5.64	0.234	1.30	0.70
	14.1	0.700	0.44	1.56

The straight line of the figure demonstrates that at pH 4.5, just as in the experiments at pH 5.3, Ag^+ is bound by egg albumin very strongly. The number of Ag-ions bound per molecule of egg albumin is 5.0, *i.e.* the same number as was found at pH 5.3. Thus these experiments, in agreement with the electrometrical determinations, show that the Ag-binding capacity of egg albumin, in contrast to that of invertase, does not vary appreciably with pH in the range mentioned. The experiments are in accord with the view that Ag is bound to egg albumin by means of the HS-groups.

Ag-binding capacity of iodine-treated egg albumin

Iodine-treated invertase (I-invertase) is very much less sensitive toward Ag-ions than is the native enzyme [2]. This strongly supports the assumption that the high Ag-binding capacity of native invertase is due chiefly to HS-groups of the enzyme which, on treatment with iodine, are oxidized to yield groups (disulfides or possibly higher oxidation products) that have no or only a weak affinity for the Ag-ion.

If the binding of Ag by egg albumin, established in the experiments described in the foregoing, is mediated chiefly by HS-groups of the protein, then it should be expected that treatment of the egg albumin with iodine would markedly decrease its Ag-binding capacity. As mentioned, Anson [14] found that the HS-groups of native egg albumin are available to iodine; iodine-treated egg albumin does not reduce ferricyanide.

A diluted solution of crystalline egg albumin was treated with iodine approximately in the same manner as that used for the oxidation of invertase. The transformation of invertase to I-invertase occurs practically instan-

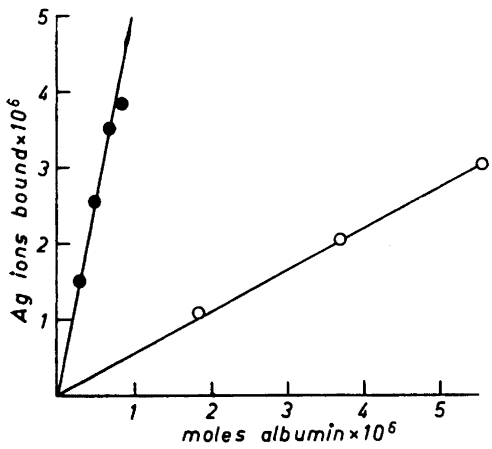

Fig. 7. Number of Ag ions in 1 000 ml bound at pH 5.3 by crystalline egg albumin (●), and by the same albumin oxidized with iodine (O).

taneously if a sufficient amount of iodine is added to the enzyme solution (pH about 5). To a certain volume of albumin solution was added a few drops of starch solution and then, dropwise, 0.01 N iodine solution until the blue colour persisted for about 30 sec. The same amount of iodine solution was then added to another albumin solution without starch. The oxidized albumin solution was freed from iodide ions by dialysis, the dry weight of the dialyzed solution was determined and suitable portions were used for the determination of the Ag-binding capacity at pH 5.3. The results are shown in Fig. 7 together with corresponding results for native egg albumin. It appears that the Ag-binding capacity of native egg albumin (5 Ag-ions per albumin molecule) has been strongly reduced by the treatment with iodine. The oxidized albumin binds less than 1 silver ion per molecule of M = 44 000. This supports the assumption that the combination of Ag with native egg albumin is mediated principally if not exclusively by the HS-groups.

DISCUSSION

A comparison of the binding of Ag^+ by yeast invertase and by egg albumin reveals several interesting facts. Both proteins bind Ag^+ very strongly, the egg albumin even more so than the enzyme; compare the straight lines of Figs. 1, 4 and 6 with the "dissociations curves" of Figs. 3 and 5. Whereas the invertase-Ag complex appears to have a dissociation constant[3,7] about 10^{-8}, the dissociation constant of the albumin-Ag compound seems to be considerably lower.

The number of Ag-ions bound by one molecule of egg albumin (M = 44 000), as determined by means of two independent methods, is 5.0—5.5. The slight deviation of the curves of Fig. 5 from the straight line may implicate that a small portion of the bound Ag is bound more loosely than the bulk which is bound very strongly. It seems probable that per molecule of egg albumin 5 Ag ions are bound by HS-groups and a further small number loosely bound by other groups, possibly imidazole groups. The weak dependence of the

silver-binding capacity of egg albumin on pH (Fig. 1) is also in accord with this view. The number of groups in egg albumin, strongly binding the silver ion, agrees with the number of HS-groups generally assumed to be present in the protein molecule. The fact that treatment of very diluted solutions of egg albumin with small amounts of iodine reduces the Ag-binding capacity to a very low value seems to show conclusively that Ag is bound to egg albumin chiefly by means of the HS-groups.

As mentioned before, previous investigations have shown the HS-groups of native egg albumin to be inaccessible to several HS-reagents, but accessible ("unmasked") after denaturation, *i.e.* an "unfolding" of the protein structure. It seems, however, that the HS-groups of the native protein react freely with silver ions, and practically no dependence of the silver-binding capacity on pH in the range investigated can be observed.

The reaction of invertase with iodine is probably accompanied by rather extensive changes in the superstructure of the protein. Such changes may explain why the activity of native invertase is not restored on treatment of I-invertase with cysteine or other reducing agents [2]. The binding of Ag^+ by invertase is not, or at least not immediately, followed by irreversible changes of the protein structure, since the inactivation of the enzyme by Ag^+ is freely reversible by H_2S, mercaptans etc., provided that the enzyme has not been incubated with the metal salt for a long time. From the behaviour of invertase in this respect no conclusion can of course be drawn regarding the behaviour of egg albumin; however, since the binding of Ag^+ by egg albumin is instantaneous and does not increase at incubation of protein and silver salt for a moderate time, it seems safe to assume that the reaction of Ag^+ with the HS-groups of egg albumin is not (rapidly) followed by destruction of the native protein structure. Thus it appears probable, that the HS-groups in native egg albumin are freely available to the silver ions without previous or simultaneous change of the protein structure.

The binding of Ag^+ by the HS-groups of egg albumin is not or only weakly dependent on pH in the range investigated. This means, that the phenomenon of a pH-dependent "masking" of the HS-groups, typical of yeast invertase, is not observed with egg albumin.

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